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(54) Title: RECOMBINANT SWINEPOX VIRUS		
(57) Abstract <p>The present invention relates to a recombinant swinepox virus capable of replication comprising foreign DNA inserted into a site in the swinepox viral DNA which is not essential for replication of the swinepox virus. The invention further relates to homology vectors which produce recombinant swinepox viruses by inserting foreign DNA into swinepox viral DNA.</p>		

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RECOMBINANT SWINEPOX VIRUS

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Within this application several publications are referenced by arabic numerals within parentheses. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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Background of the Invention

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Swinepox virus (SPV) belongs to the family Poxviridae. Viruses belonging to this group are large, double-stranded DNA viruses that characteristically develop in the cytoplasm of the host cell. SPV is the only member of the genus Suipoxvirus. Several features distinguish SPV from other poxviruses. SPV exhibits species specificity (18) compared to other poxviruses such as vaccinia which exhibit a broad host range. SPV infection of tissue culture cell lines also differs dramatically from other poxviruses (24). It has also been demonstrated that SPV does not exhibit antigenic cross-reactivity with vaccinia virus and shows no gross detectable homology at the DNA level with the ortho, lepori, avi or entomopox virus groups (24). Accordingly, what is known and described in the prior art regarding other poxviruses does not pertain a priori to swinepox virus.

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SPV is only mildly pathogenic, being characterized by a self-limiting infection with lesions detected only in the

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skin and regional lymph nodes. Although the SPV infection is quite limited, pigs which have recovered from SPV are refractory to challenge with SPV, indicating development of active immunity (18).

5 The present invention concerns the use of SPV as a vector for the delivery of vaccine antigens and therapeutic agents to swine. The following properties of SPV support this rationale: SPV is only mildly pathogenic in swine, 10 SPV is species specific, and SPV elicits a protective immune response. Accordingly, SPV is an excellent candidate for a viral vector delivery system, having little intrinsic risk which must be balanced against the benefit contributed by the vector's vaccine and 15 therapeutic properties.

The prior art for this invention stems first from the ability to clone and analyze DNA while in bacterial plasmids. The techniques that are available are detailed 20 for the most part in Maniatis et al., 1983 and Sambrook et al., 1989. These publications teach state of the art general recombinant DNA techniques.

25 Among the poxviruses, five (vaccinia, fowlpox, canarypox, pigeon, and raccoon pox) have been engineered, previous to this disclosure, to contain foreign DNA sequences. Vaccinia virus has been used extensively to vector foreign genes (25) and is the subject of U.S. Patents 4,603,112 and 4,722,848. Similarly, fowlpox has been 30 used to vector foreign genes and is the subject of several patent applications EPA 0 284 416, PCT WO 89/03429, and PCT WO 89/12684. Raccoon pox (10) and Canarypox (31) have been utilized to express antigens from the rabies virus. These xamples of insertions of 35 foreign genes into poxviruses do not include an example

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from the genus *Suipoxvirus*. Thus, they do not teach methods to genetically engineer swinepox viruses, that is, where to make insertions and how to get expression in swinepox virus.

5 The idea of using live viruses as delivery systems for antigens has a very long history going back to the first live virus vaccines. The antigens delivered were not foreign but were naturally expressed by the live virus in
10 the vaccines. The use of viruses to deliver foreign antigens in the modern sense became obvious with the recombinant vaccinia virus studies. The vaccinia virus was the vector and various antigens from other disease causing viruses were the foreign antigens, and the
15 vaccine was created by genetic engineering. While the concept became obvious with these disclosures, what was not obvious was the answer to a more practical question of what makes the best candidate virus vector. In answering this question, details of the pathogenicity of
20 the virus, its site of replication, the kind of immune response it elicits, the potential it has to express foreign antigens, its suitability for genetic engineering, its probability of being licensed by regulatory agencies, etc, are all factors in the
25 selection. The prior art does not teach these questions of utility.

The prior art relating to the use of poxviruses to deliver therapeutic agents relates to the use of a
30 vaccinia virus to deliver interleukin-2 (12). In this case, although the interleukin-2 had an attenuating effect on the vaccinia vector, the host did not demonstrate any therapeutic benefit.

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The therapeutic agent that is delivered by a viral vector of the present invention must be a biological molecule that is a by-product of swinepox virus replication. This limits the therapeutic agent in the first analysis to either DNA, RNA or protein. There are examples of therapeutic agents from each of these classes of compounds in the form of anti-sense DNA, anti-sense RNA (16), ribozymes (34), suppressor tRNAs (2), interferon-inducing double stranded RNA and numerous examples of protein therapeutics, from hormones, e.g., insulin, to lymphokines, e.g., interferons and interleukins, to natural opiates. The discovery of these therapeutic agents and the elucidation of their structure and function does not make obvious the ability to use them in a viral vector delivery system.

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Summary of the Invention

5 The invention provides a recombinant swinepox virus capable of replication which comprises swinepox viral DNA and foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox virus is introduced. The foreign DNA is inserted into the swinepox viral DNA at a site which is not essential for replication of the swinepox virus and is under the control of a promoter.

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This invention provides a homology vector for producing a recombinant swinepox virus by inserting foreign DNA into the genomic DNA of a swinepox virus which comprises a double-stranded DNA molecule. This molecule consists essentially of double-stranded foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox virus is introduced. At one end of this foreign DNA is double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox virus. At the other end of the foreign DNA is double-stranded swinepox viral DNA homologous to genomic DNA located at the other side of the same site on the genomic DNA.

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Brief Description of the Figures

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- Figure 1 Details of the SPV Kasza Strain. Diagram of SPV genomic DNA showing the unique long and Terminal repeat (TR) regions. A restriction map for the enzyme *HindIII* is indicated (23). Fragments are lettered in order of decreasing size. Note that the terminal repeats are greater than 2.1 kb but less than 9.7 kb in size.
- Figure 2 DNA sequence from homology vector 515-85.1. The sequence of two regions of the homology vector 515-85.1 are shown. The first region (Figure 2A) (SEQ ID NO:1) covers a 599 base pair sequence which flanks the unique *AccI* site as indicated in Figure 3. The beginning (Met) and end (Val) of a 115 amino acid ORF is indicated by the translation of amino acids below the DNA sequence. The second region (Figure 2B) (SEQ ID NO:3) covers the 899 base pairs upstream of the unique *HindIII* site as indicated in Figure 3. The beginning (Asp) and end (Ile) of a 220 amino acid ORF is indicated by the translation of amino acids below the DNA sequence.
- Figure 3 Homology between the 515.85.1 ORF and the Vaccinia virus 01L ORF. The first line shows a restriction map of the SPV *HindIII* M fragment. The second map shows a restriction map of the DNA insertion in plasmid 515-85.1. The location of the 515-85.1 [VV 01L-like] ORF is indicated on the map. The locations of the DNA sequences shown in Figure 2 are indicated below the map by heavy bars. The third line shows the homology between the VV 01L ORF (SEQ

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ID NO:5) and the 515-85.1 ORF (SEQ ID NO:6) at their respective N-termini. The fourth line shows the homology between the VV 01L ORF (SEQ ID NO:7) and the 515-85.1 ORF (SEQ ID NO:8) at their respective C-termini.

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Figure 4 Detailed description of the DNA insertion in Homology Vector 520-17.5. Diagram showing the orientation of DNA fragments assembled in plasmid 520-17.5. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown (SEQ ID NO's: 9, 10, 13, and 16). The restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements are also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), early promoter 1 (EP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and *Escherichia coli* (*E. coli*).

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Figure 5 Detailed description of the DNA insertion in Homology Vector 538-46.16. Diagram showing the orientation of DNA fragments assembled in plasmid 538-46.16. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments are also shown (SEQ ID NO's: 17, 18, 21, 26, and 28). The restriction sites used to

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5 generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), pseudorabies virus (PRV), glycoprotein 50 (gp50), glycoprotein 63 (gp63), early promoter 1 (EP1), late promoter 1 (LP1), late promoter 2 (LP2), lactose operon Z gene (lacZ), and *Escherichia coli* (E. coli).

20 Figure 6 Western blot of lysates from recombinant SPV infected cells with anti-serum to PRV. Lanes (A) uninfected Vero cell lysate, (B) S-PRV-000 (pseudorabies virus Cooper strain) infected cell lysate, (C) pre-stained molecular weight markers, (D) uninfected EMSK cell lysate, (E) S-SPV-000 infected cell lysate, (F) S-SPV-003 infected cell lysate, (G) S-SPV-008 infected cell lysate. Cell lysates were prepared as described in the PREPARATION OF INFECTED CELL LYSATES. Approximately 1/5 of the total lysate sample was loaded in each lane.

35 Figure 7 DNA sequence of NDV Hemagglutinin-Neuraminidase gene (HN) (SEQ ID NO: 29). The sequence of 1907 base pairs of the NDV HN cDNA clone are shown. The translational start and stop of the HN gene is indicated by the amino acid translation below the DNA sequence.

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Figure 8 Detailed description of the DNA insertion in Homology Vector 538-46.26. Diagram showing the orientation of DNA fragments assembled in plasmid 538-46.26. The origin of each fragment is indicated in the table. The sequences located at each of the junctions between fragments is also shown (SEQ ID NO's: 31, 32, 34, 37, and 40). The restriction sites used to generate each fragment as well as the synthetic linker sequences which were used to join the fragments are described for each junction. The synthetic linker sequences are underlined by a heavy bar. The location of several gene coding regions and regulatory elements is also given. The following two conventions are used: numbers in parenthesis () refer to amino acids, and restriction sites in brackets [] indicate the remnants of sites which were destroyed during construction. The following abbreviations are used, swinepox virus (SPV), Newcastle Disease virus (NDV), hemagglutinin-neuraminidase (HN), early promoter 1 (EP1), late promoter 1 (LP1), late promoter 2 (LP2), lactose operon 2 gene (lacZ), and *Escherichia coli* (*E. coli*).

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Detailed Description Of The Invention

5 The present invention provides a recombinant swinepox virus (SPV) capable of replication in an animal into which the recombinant swinepox virus is introduced which comprises swinepox viral DNA and foreign DNA encoding RNA which does not naturally occur in the animal into which the recombinant swinepox virus is introduced, the foreign DNA being inserted into the swinepox viral DNA at an
10 insertion site which is not essential for replication of the swinepox virus and being under the control of a promoter.

15 For purposes of this invention, "a recombinant swinepox virus capable of replication" is a live swinepox virus which has been generated by the recombinant methods well known to those of skill in the art, e.g., the methods set forth in HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV in Materials and Methods and
20 has not had genetic material essential for the replication of the recombinant swinepox virus deleted.

25 For purposes of this invention, "an insertion site which is not essential for replication of the swinepox virus" is a location in the genome where a sequence of DNA is not necessary for viral replication, for example, complex protein binding sequences, sequences which code for reverse transcriptase or an essential glycoprotein, DNA sequences necessary for packaging, etc.

30 For purposes of this invention, a "promoter" is a specific DNA sequence on the DNA molecule to which the foreign RNA polymerase attaches and at which transcription of the foreign RNA is initiated.

35 The invention further provides foreign RNA which encodes a polypeptide. Preferably, the polypeptide is antigenic in the animal.

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Preferably, this antigenic polypeptide is a linear polymer of more than 10 amino acids linked by peptide bonds which stimulates the animal to produce antibodies.

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The invention further provides an insertion site present within the larger *Hind*III to *Bgl*II subfragment of the *Hind*III M fragment of swinepox viral DNA. Preferably, the insertion site is within an open reading frame contained in the *Hind*III to *Bgl*II subfragment. Preferably, the insertion site is the *Acc*I restriction endonuclease site located in the *Hind*III to *Bgl*II subfragment.

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15 The invention further provides an insertion site within an open reading frame encoding swinepox thymidine kinase.

For purposes of this invention, an "open reading frame" is a segment of DNA which contains codons that can be transcribed into RNA which can be translated into an amino acid sequence and which does not contain a termination codon.

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The invention further provides a recombinant swinepox virus comprising foreign DNA encoding RNA encoding an antigenic polypeptide which is or is from pseudorabies virus (PRV) glycoprotein 50, pseudorabies virus (PRV) glycoprotein II, Pseudorabies virus (PRV) glycoprotein III, Pseudorabies virus (PRV) glycoprotein H, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hydodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic polypeptide is Pseudorabies Virus (PRV) glycoprotein 50. Preferably, the antigenic

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protein is Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase.

5 The invention further provides a recombinant swinepox virus comprising foreign DNA encoding RNA encoding an antigenic polypeptide which is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus, Swine Influenza Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*.

10 The invention further provides a recombinant swinepox virus where the foreign DNA encodes RNA which encodes a polypeptide which is a detectable marker. Preferably the detectable marker is the polypeptide *E. coli* β -galactosidase. For purposes of this invention, a
15 "polypeptide which is a detectable marker" includes the bimer, trimer and tetramer form of the polypeptide. *E. coli* β -galactosidase is a tetramer composed of four polypeptides or monomer sub-units. Preferably, this
20 recombinant swinepox virus is designated S-SPV-003 (ATCC Accession No. VR 2335). The S-SPV-003 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes
25 of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2335.

30 The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding RNA encoding the antigenic polypeptide pseudorabies virus (PRV) glycoprotein 50 further comprising foreign DNA encoding a polypeptide which is a detectable marker. Preferably, this recombinant swinepox
35 virus is designated S-SPV-008 (ATCC Accession No. VR 2339). The S-SPV-008 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent

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Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2339.

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The invention further provides for a recombinant swinepox virus capable of replication which contains foreign DNA encoding RNA encoding the antigenic polypeptide Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase further comprising foreign DNA encoding a polypeptide which is a detectable marker. Preferably, this recombinant swinepox virus is designated S-SPV-009 (ATCC Accession No. VR 2344). The S-SPV-009 swinepox virus has been deposited pursuant to the Budapest Treaty on the International Deposit of Microorganisms for the Purposes of Patent Procedure with the Patent Culture Depository of the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A. under ATCC Accession No. VR 2344.

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The invention further provides that the inserted foreign DNA is under the control of a promoter. Preferably, the promoter is a swinepox viral promoter. Preferably, the promoter is a synthetic pox viral promoter. For purposes of this invention, the promoters were generated by methods well known to those of skill in the art, for example, as set forth in the STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS in Materials and Methods. For purposes of this invention, a synthetic pox promoter includes a synthetic late pox promoter, a synthetic early pox promoter or a synthetic early/late pox promoter.

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The invention provides for a homology vector for producing a recombinant swinepox virus by inserting foreign DNA into the genomic DNA of a swinepox virus. The homology vector comprises a double-stranded DNA molecule consisting essentially of a double-stranded

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foreign DNA encoding RNA which does not naturally occur in an animal into which the recombinant swinepox virus is introduced, with at one end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the swinepox virus, and at the other end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at the other side of the same site on the genomic DNA. Preferably, the RNA encodes a polypeptide.

In one embodiment, the polypeptide is antigenic in the animal. Preferably, the antigenic polypeptide is or is from pseudorabies virus (PRV) glycoprotein 50, pseudorabies virus (PRV) glycoprotein II, Pseudorabies virus (PRV) glycoprotein III, Pseudorabies virus (PRV) glycoprotein H, Transmissible gastroenteritis (TGE) glycoprotein 195, Transmissible gastroenteritis (TGE) matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hyodysenteriae* protective antigen, Bovine Viral Diarrhea (BVD) glycoprotein 55, Newcastle Disease Virus (NDV) hemagglutinin-neuraminidase, swine flu hemagglutinin or swine flu neuraminidase. Preferably, the antigenic polypeptide is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus, Swine Influenza Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*.

In one embodiment, the polypeptide is a detectable marker. Preferably, the polypeptide which is a detectable marker is *E. coli* β -galactosidase.

In one embodiment of the invention, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the larger *Hind*III to *Bgl*II subfragment of the *Hind*III M fragment of swinepox virus. Preferably, the double-stranded swinepox viral DNA is homologous to

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genomic DNA present within the open reading frame contained in this *Hind*III to *Bgl*III subfragment. Preferably, the double-stranded swinepox viral DNA is homologous to genomic DNA present within the *Acc*I restriction endonuclease site located in this *Hind*III to *Bgl*III subfragment.

For purposes of this invention, a "homology vector" is a plasmid constructed to insert foreign DNA in a specific site on the genome of a swinepox virus.

In one embodiment of the invention, the double-stranded swinepox viral DNA in the homology vector is homologous to genomic DNA present within the open reading frame encoding swinepox thymidine kinase.

The invention further provides a homology vector where foreign DNA further comprises a synthetic pox viral promoter.

The invention further provides a vaccine which comprises an effective immunizing amount of a recombinant swinepox virus of the present invention and a suitable carrier.

Suitable carriers for the pseudorabies virus are well known in the art and include proteins, sugars, etc. One example of such a suitable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc.

For purposes of this invention, an "effective immunizing amount" of the recombinant swinepox virus of the present invention is within the range of 10^3 to 10^9 PFU/dose.

The present invention also provides a method of immunizing an animal, wherein the animal is a swine,

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bovine, equine, caprine or ovine. For purposes of this invention, this includes immunizing the animal against the virus or viruses which cause the disease or diseases pseudorabies, transmissible gastroenteritis, swine rotavirus, swine parvovirus, *Serpulina hyodysenteriae*, bovine viral diarrhea, Newcastle disease, swine flu, foot and mouth disease, hog cholera, African swine fever or *Mycoplasma hyopneumoniae*. The method comprises administering to the animal an effective immunizing dose of the vaccine of the present invention. The vaccine may be administered by any of the methods well known to those skilled in the art, for example, by intramuscular, subcutaneous, intraperitoneal or intravenous injection. Alternatively, the vaccine may be administered intranasally or orally.

The present invention also provides a method for testing a swine to determine whether the swine has been vaccinated with the vaccine of the present invention, particularly the embodiment which contains the recombinant swinepox virus S-SPV-008 (ATCC Accession No. VR 2339), or is infected with a naturally-occurring, wild-type pseudorabies virus. This method comprises obtaining from the swine to be tested a sample of a suitable body fluid, detecting in the sample the presence of antibodies to pseudorabies virus, the absence of such antibodies indicating that the swine has been neither vaccinated nor infected, and for the swine in which antibodies to pseudorabies virus are present, detecting in the sample the absence of antibodies to pseudorabies virus antigens which are normally present in the body fluid of a swine infected by the naturally-occurring pseudorabies virus but which are not present in a vaccinated swine indicating that the swine was vaccinated and is not infected.

The present invention also provides a host cell infected with a recombinant swinepox virus capable of replication.

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In one embodiment, the host cell is a mammalian cell. Preferably, the mammalian cell is a Vero cell. Preferably, the mammalian cell is an EMSK cell.

5 For purposes of this invention a "host cell" is a cell used to propagate a vector and its insert. Infecting the cells was accomplished by methods well known to those of skill in the art, for example, as set forth in INFECTION - TRANSFECTION PROCEDURE in Material and Methods.

10 Methods for constructing, selecting and purifying recombinant swinepox virus, including S-SPV-003, S-SPV-008 and S-SPV-009, are detailed below in Materials and Methods.

Materials and Methods

5 PREPARATION OF SWINEPOX VIRUS STOCK SAMPLES. Swinepox virus (SPV) samples were prepared by infecting embryonic swine kidney (EMSK) cells at a multiplicity of infection of 0.01 PFU/cell in a 1:1 mixture of Iscove's Modified Dulbecco's Medium (IMDM) and RPMI 1640 medium containing 2 mM glutamine, 100 units/ml penicillin, 100 units/ml streptomycin (these components were obtained from Sigma or equivalent supplier, and hereafter are referred to as EMSK negative medium). Prior to infection, the cell monolayers were washed once with EMSK negative medium to remove traces of fetal bovine serum. The SPV contained in the initial inoculum (0.5 ml for 10 cm plate; 10 ml for T175 cm flask) was then allowed to absorb onto the cell monolayer for two hours, being redistributed every half hour. After this period, the original inoculum was brought up to the recommended volume with the addition of complete EMSK medium (EMSK negative medium plus 5% fetal bovine serum). The plates were incubated at 37°C in 5% CO₂ until cytopathic effect was complete. The medium and cells were harvested and frozen in a 50 ml conical screw cap tube at -70°C. Upon thawing at 37°C, the virus stock was aliquoted into 1.0 ml vials and refrozen at -70°C. The titers were usually about 10⁶ PFU/ml.

30 PREPARATION OF SPV DNA. For swinepox virus DNA isolation, a confluent monolayer of EMSK cells in a T175 cm² flask was infected at a multiplicity of 0.1 and incubated 4-6 days until the cells were showing 100% cytopathic effect. The infected cells were then harvested by scraping the cells into the medium and centrifuging at 3000 rpm for 5 minutes in a clinical centrifuge. The medium was decanted, and the cell pellet was gently resuspended in 1.0 ml Phosphate Buffer Saline (PBS: 1.5g Na₂HPO₄, 0.2g KH₂PO₄, 0.8g NaCl and 0.2g KCl per liter H₂O) (per T175) and subjected to two successive freeze-thaws (-70° C to 37° C). Upon the last thaw, the cells (on ice) were

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sonicated two times for 30 seconds each with 45 seconds cooling time in between. Cellular debris was then removed by centrifuging (Sorvall RC-5B superspeed centrifuge) at 3000 rpm for 5 minutes in a HB4 rotor at 4° C. SPV virions, present in the supernatant, were then pelleted by centrifugation at 15,000 rpm for 20 minutes at 4° C in a SS34 rotor (Sorvall) and resuspended in 10 mM Tris (pH 7.5). This fraction was then layered onto a 36% sucrose gradient (w/v in 10 mM tris pH 7.5) and centrifuged (Beckman L8-70M Ultracentrifuge) at 18,000 rpm for 60 minutes in a SW41 rotor (Beckman) at 4° C. The virion pellet was resuspended in 1.0 ml of 10 mM tris pH 7.5 and sonicated on ice for 30 seconds. This fraction was layered onto a 20% to 50% continuous sucrose gradient and centrifuged 16,000 rpm for 60 minutes in a SW41 rotor at 4° C. The SPV virion band located about three quarters down the gradient was harvested, diluted with 20% sucrose and pelleted by centrifugation at 18,000 rpm for 60 minutes in a SW41 rotor at 4° C. The resultant pellet was then washed once with 10 mM Tris pH 7.5 to remove traces of sucrose and finally resuspended in 10 mM Tris pH 7.5. SPV DNA was then extracted from the purified virions by lysis (4 hours at 60° C) induced by the addition of EDTA, SDS, and proteinase K to final concentrations of 20 mM, 0.5% and 0.5 mg/ml, respectively. After digestion, three phenol:chloroform (1:1) extractions were conducted and the sample precipitated by the addition of two volumes of absolute ethanol and incubation at -20° C for 30 minutes. The sample was then centrifuged in an Eppendorf minifuge for 5 minutes at full speed. The supernatant was decanted, and the pellet air dried and rehydrated in 0.01 M Tris pH 7.5, 1 mM EDTA at 4° C.

PREPARATION OF INFECTED CELL LYSATES. For cell lysate preparation, serum free medium was used. A confluent monolayer of cells (EMSK for SPV or VERO for PRV) in a 25 cm² flask or a 60 mm petri dish was infected with 100 µl of virus sample. After cytopathic effect was complete,

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the medium and cells were harvested and the cells were pelleted at 3000 rpm for 5 minutes in a clinical centrifuge. The cell pellet was resuspended in 250 μ l of disruption buffer (2% sodium dodecyl sulfate, 2% β -mercapto-ethanol). The samples were sonicated for 30 seconds on ice and stored at -20°C .

WESTERN BLOTTING PROCEDURE. Samples of lysates and protein standards were run on a polyacrylamide gel according to the procedure of Laemmli (1970). After gel electrophoresis the proteins were transferred and processed according to Sambrook et al. (1982). The primary antibody was a swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) diluted 1:100 with 5% non-fat dry milk in Tris-sodium chloride, and sodium Azide (TSA: 6.61g Tris-HCl, 0.97g Tris-base, 9.0g NaCl and 2.0g Sodium Azide per liter H_2O). The secondary antibody was a goat anti-swine alkaline phosphatase conjugate diluted 1:1000 with TSA.

MOLECULAR BIOLOGICAL TECHNIQUES. Techniques for the manipulation of bacteria and DNA, including such procedures as digestion with restriction endonucleases, gel electrophoresis, extraction of DNA from gels, ligation, phosphorylation with kinase, treatment with phosphatase, growth of bacterial cultures, transformation of bacteria with DNA, and other molecular biological methods are described by Maniatis et al. (1982) and Sambrook et al. (1989). Except as noted, these were used with minor variation.

DNA SEQUENCING. Sequencing was performed using the USB Sequenase Kit and ^{35}S -dATP (NEN). Reactions using both the dGTP mix s and the dTTP mixes were performed to clarify areas of compression. Alternatively, compressed areas were resolved on formamide gels. Templates were double-stranded plasmid subclones or single stranded M13 subclones, and primers were either made to the vector

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just outside the insert to be sequenced, or to previously obtained sequence. Sequence obtained was assembled and compared using Dnastar software. Manipulation and comparison of sequences obtained was performed with SupercloneTM and SuperseeTM programs from Coral Software.

CLONING WITH THE POLYMERASE CHAIN REACTION. The polymerase chain reaction (PCR) was used to introduce restriction sites convenient for the manipulation of various DNAs. The procedures used are described by Innis, et al. (1990). In general, amplified fragments were less than 500 base pairs in size and critical regions of amplified fragments were confirmed by DNA sequencing. The primers used in each case are detailed in the descriptions of the construction of homology vectors below.

HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. This method relies upon the homologous recombination between the swinepox virus DNA and the plasmid homology vector DNA which occurs in the tissue culture cells containing both swinepox virus DNA and transfected plasmid homology vector. For homologous recombination to occur, the monolayers of EMSK cells are infected with S-SPV-001 (Kasza SPV strain, 17) at a multiplicity of infection of 0.01 PFU/cell to introduce replicating SPV (i.e. DNA synthesis) into the cells. The plasmid homology vector DNA is then transfected into these cells according to the INFECTION - TRANSFECTION PROCEDURE. The construction of homology vectors used in this procedure is described below

INFECTION - TRANSFECTION PROCEDURE. 6 cm plates of EMSK cells (about 80% confluent) were infected with S-SPV-001 at a multiplicity of infection of 0.01 PFU/cell in EMSK negativ medium and incubated at 37°C in a humidified 5% CO₂ environment for 5 hours. The transfection procedure used is essentially that recommended for LipofectinTM

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Reagent (BRL). Briefly, for each 6 cm plate, 15 μ g of plasmid DNA was diluted up to 100 μ l with H₂O. Separately, 50 micrograms of Lipofectin Reagent was diluted to 100 μ l with H₂O. The 100 μ l of diluted Lipofectin Reagent was then added dropwise to the diluted plasmid DNA contained in a polystyrene 5 ml snap cap tube and mixed gently. The mixture was then incubated for 15-20 minutes at room temperature. During this time, the virus inoculum was removed from the 6 cm plates and the cell monolayers washed once with EMSK negative medium. Three ml of EMSK negative medium was then added to the plasmid DNA/lipofectin mixture and the contents pipetted onto the cell monolayer. The cells were incubated overnight (about 16 hours) at 37°C in a humidified 5% CO₂ environment. The next day the 3 ml of EMSK negative medium was removed and replaced with 5 ml EMSK complete medium. The cells were incubated at 37°C in 5% CO₂ for 3-7 days until cytopathic effect from the virus was 80-100%. Virus was harvested as described above for the preparation of virus stocks. This stock was referred to as a transfection stock and was subsequently screened for recombinant virus by the BLUOGAL SCREEN FOR RECOMBINANT SWINEPOX VIRUS OR CPRG SCREEN FOR RECOMBINANT SWINEPOX VIRUS.

SCREEN FOR RECOMBINANT SPV EXPRESSING B-GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). When the *E. coli* β -galactosidase (*lacZ*) marker gene was incorporated into a recombinant virus the plaques containing the recombinants were visualized by one of two simple methods. In the first method, the chemical BluogalTM (Bethesda Research Labs) was incorporated (200 μ g/ml) into the agarose overlay during the plaque assay, and plaques expressing active β -galactosidase turned blue. The blue plaques were then picked onto fresh cells (EMSK) and purified by further blue plaque isolation. In the second method, CPRG (Boehringer Mannheim) was incorporated (400 μ g/ml) into the agarose overlay during the plaque assay, and plaques

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expressing active β -galactosidase turned red. The red plaques were then picked onto fresh cells (EMSK) and purified by further red plaque isolation. In both cases viruses were typically purified with three rounds of plaque purification.

5

SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV USING BLACK PLAQUE ASSAYS. To analyze expression of foreign antigens expressed by recombinant swinepox

10 viruses, monolayers of EMSK cells were infected with recombinant SPV, overlaid with nutrient agarose media and incubated for 6-7 days at 37°C for plaque development to occur. The agarose overlay was then removed from the dish, the cells fixed with 100% methanol for 10 minutes

15 at room temperature and the cells air dried. Fixation of the cells results in cytoplasmic antigen as well as surface antigen detection whereas specific surface antigen expression can be detected using non-fixed cells. The primary antibody was then diluted to the appropriate

20 dilution with PBS and incubated on the cell monolayer for 2 hours at room temperature. To detect PRV gp50 expression from S-SPV-008, swine anti-PRV serum (Shope strain; lot370, PDV8201, NVSL, Ames, IA) was used (diluted 1:100). To detect NDV HN expression from S-SPV-

25 009, a rabbit antiserum specific for the HN protein (rabbit anti-NDV#2) was used (diluted 1:1000). Unbound antibody was then removed by washing the cells three times with PBS at room temperature. The secondary antibody, either a goat anti-swine (PRVgp50; S-SPV-008)

30 or goat anti-rabbit (NDV HN; S-SPV-009), horseradish peroxidase conjugate was diluted 1:250 with PBS and incubated with the cells for 2 hours at room temperature. Unbound secondary antibody was then removed by washing the cells three times with PBS at room temperature. The

35 cells were then incubated 15-30 minutes at room temperature with freshly prepared substrate solution (100 μ g/ml 4-chloro-1-naphthol, 0.003% H_2O_2 in PBS). Plaques expressing the correct antigen stain black.

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STRATEGY FOR THE CONSTRUCTION OF SYNTHETIC POX VIRAL PROMOTERS. For recombinant swinepox vectors synthetic pox promoters offer several advantages including the ability to control the strength and timing of foreign gene expression. We chose to design three promoter cassettes LP1, EP1 and LP2 based on promoters that have been defined in the vaccinia virus (1, 7 and 8). Each cassette was designed to contain the DNA sequences defined in vaccinia flanked by restriction sites which could be used to combine the cassettes in any order or combination. Initiator methionines were also designed into each cassette such that inframe fusions could be made at either EcoRI or BamHI sites. A set of translational stop codons in all three reading frames and an early transcriptional termination signal (9) were also engineered downstream of the inframe fusion site. DNA encoding each cassette was synthesized according to standard techniques and cloned into the appropriate homology vectors (see Figures 4, 5 and 8).

HOMOLOGY VECTOR 515-85.1. The plasmid 515-85.1 was constructed for the purpose of inserting foreign DNA into SPV. It contains a unique AccI restriction enzyme site into which foreign DNA may be inserted. When a plasmid, containing a foreign DNA insert at the AccI site, is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing the foreign DNA will result. A restriction map of the DNA insert in homology vector 515-85.1 is given in figure 4. It may be constructed utilizing standard recombinant DNA techniques (22 and 29), by joining two restriction fragments from the following sources. The first fragment is an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). The second fragment is an approximately 3300 base pair *Hind*III to *Bgl*II restriction sub-fragment of the SPV *Hind*III restriction fragment M (23).

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HOMOLOGY VECTOR 520-17.5. The plasmid 520-17.5 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene flanked by SPV DNA. Upstream of the marker gene is an approximately 2015 base pair fragment of SPV DNA. Downstream of the marker gene is an approximately 1103 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the marker gene will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic early/late pox promoter. A detailed description of the plasmid is given in figure 4. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 4. The plasmid vector is derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2015 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3010 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1103 base pair *Acc*I to *Bgl*II restriction sub-fragment of the SPV *Hind*III fragment M (23).

HOMOLOGY VECTOR 538-46.16. The plasmid 538-46.16 was constructed for the purpose of inserting foreign DNA into SPV. It incorporates an *E. coli* β -galactosidase (*lacZ*) marker gene and the PRV gp50 gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 2015 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1103 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the foreign genes will result. Note that the β -galactosidase

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(lacZ) marker gene is under the control of a synthetic late pox promoter and the gp50 gene is under the control of a synthetic early/late pox promoter. A detailed description of the plasmid is given in figure 5. It may be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 5. The plasmid vector is derived from an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2015 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 3010 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751 (11). Fragment 3 is an approximately 1571 base pair *Eco*RI to *Stu*I restriction sub-fragment of the PRV *Bam*HI fragment 7 (21). Note that the *Eco*RI site was introduced in to this fragment by PCR cloning. In this procedure the primers described below were used along with a template consisting of a PRV *Bam*HI #7 fragment subcloned into pSP64. The first primer 87.03 (5'-CGCGAATTCGCTCGCAGCGCTATTGGC-3') (SEQ ID NO:41) sits down on the PRV gp50 sequence (26) at approximately amino acid 3 priming toward the 3' end of the gene. The second primer 87.06 (5'-GTAGGAGTGGCTGCTGAAG-3') (SEQ ID NO:42) sits down on the opposite strand at approximately amino acid 174 priming toward the 5' end of the gene. The PCR product may be digested with *Eco*RI and *Sal*I to produce an approximately 509 base pair fragment. The approximately 1049 base pair *Sal*I to *Stu*I sub-fragment of PRV *Bam*HI #7 may then be ligated to the approximately 509 base pair *Eco*RI to *Sal*I fragment to generate the approximately 1558 base pair *Eco*RI to *Stu*I fragment 3. Fragment 4 is an approximately 1103 base pair *Acc*I to *Bgl*II restriction sub-fragment of the SPV *Hind*III fragment M (23).

HOMOLOGY VECTOR 538-46.26. The plasmid 538-46.26 was constructed for the purpose of inserting foreign DNA into

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SPV. It incorporates an *E.coli* β -galactosidase (*lacZ*) marker gene and the Newcastle Disease Virus (NDV) hemagglutinin-Neuraminidase (HN) gene flanked by SPV DNA. Upstream of the foreign genes is an approximately 2015
5 base pair fragment of SPV DNA. Downstream of the foreign genes is an approximately 1103 base pair fragment of SPV DNA. When this plasmid is used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing DNA coding for the
10 foreign genes will result. Note that the β -galactosidase (*lacZ*) marker gene is under the control of a synthetic late pox promoter and the HN gene is under the control of a synthetic early/late pox promoter. A detailed description of the plasmid is given in figure 8. It may
15 be constructed utilizing standard recombinant DNA techniques (22 and 30), by joining restriction fragments from the following sources with the synthetic DNA sequences indicated in figure 8. The plasmid vector is derived from an approximately 2972 base pair *Hind*III to
20 *Bam*HI restriction fragment of pSP64 (Promega). Fragment 1 is an approximately 2015 base pair *Hind*III to *Acc*I restriction sub-fragment of the SPV *Hind*III restriction fragment M (23). Fragment 2 is an approximately 1810 base pair *Ava*II to *Nae*I restriction fragment of a NDV HN
25 cDNA clone. The sequence of the HN cDNA clone is given in figure 7. The cDNA clone was generated from the B1 strain of NDV using standard cDNA cloning techniques (14). Fragment 3 is an approximately 3010 base pair *Bam*HI to *Pvu*II restriction fragment of plasmid pJF751
30 (11). Fragment 4 is an approximately 1103 base pair *Acc*I to *Bgl*II restriction sub-fragment of the SPV *Hind*III fragment M (23).

HOMOLOGY VECTOR 520-90.15. The plasmid 520-90.15 was
35 constructed for the purpose of inserting foreign DNA into SPV. It contains a unique *Nde*I restriction enzyme site into which foreign DNA may be inserted. When a plasmid, containing a foreign DNA insert at the *Nde*I site, is used

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according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV a virus containing the foreign DNA will result. Plasmid 520-90.15 was constructed utilizing standard recombinant DNA techniques (22 and 5 30), by joining two restriction fragments from the following sources. The first fragment is an approximately 2972 base pair *Hind*III to *Bam*HI restriction fragment of pSP64 (Promega). The second fragment is an approximately 1700 base pair *Hind*III to *Bam*HI restriction 10 subfragment of the SPV *Hind*III restriction fragment G (23).

Examples

Example 1

15 Homology Vector 515-85.1

The homology vector 515-85.1 is a plasmid useful for the insertion of foreign DNA into SPV. Plasmid 515-85.1 contains a unique *Acc*I restriction site into which 20 foreign DNA may be cloned. A plasmid containing such a foreign DNA insert may be used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate a SPV containing the foreign DNA. For this procedure to be successful it is important 25 that the insertion site (*Acc*I) be in a region non-essential to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. We have demonstrated that the *Acc*I site in 30 homology vector 515-85.1 may be used to insert foreign DNA into at least three recombinant SPV (see examples 2-4).

In order to define an appropriate insertion site, a 35 library of SPV *Hind*III restriction fragments was generated. Several of these restriction fragments (*Hind*III fragments G, J, and M see figure 1) were subjected to restriction mapping analysis. Two

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restriction sites were identified in each fragment as potential insertion sites. These sites included *HpaI* and *NruI* in fragment G, *BalI* and *XbaI* in fragment J, and *AccI* and *PstI* in fragment M. A β -galactosidase (*lacZ*) marker gene was inserted in each of the potential sites. The resulting plasmids were utilized in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The generation of recombinant virus was determined by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE ASSAYS. Four of the six sites were found to generate recombinant virus, however the ability of each of these viruses to be purified away from the parental SPV varied greatly. In one case virus could not be purified above the level of 1%, in another case virus could not be purified above the level of 50%, and in a third case virus could not be purified above the level of 90%. The inability to purify these viruses indicates instability at the insertion site. This makes the corresponding sites inappropriate for insertion of foreign DNA. However the insertion at one site, the *AccI* site of Homology vector 515-85.1, resulted in a virus which was easily purified to 100% (see example 2), clearly defining an appropriate site for the insertion of foreign DNA.

The homology vector 515-85.1 was further characterized by DNA sequence analysis. Two regions of the homology vector were sequenced. The first region covers a 599 base pair sequence which flanks the unique *AccI* site (see figure 2). The second region covers the 899 base pairs upstream of the unique *HindIII* site (see figure 2). The sequence of the first region codes for an open reading frame (ORF) which shows homology to amino acids 1 to 115 of the vaccinia virus (VV) O1L open reading frame identified by Goebel et al, 1990 (see figure 3). The sequence of the second region codes for an open reading frame which shows homology to amino acids 568 to 666 of the same vaccinia virus O1L open reading frame (see figure 3). These data suggest that the *AccI* site

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interrupts the presumptive VV 01L-like ORF at approximately amino acid 41, suggesting that this ORF codes for a gene non-essential for SPV replication. Goebel et al. suggest that the VV 01L ORF contains a leucine zipper motif characteristic of certain eukaryotic transcriptional regulatory proteins, however they indicate that it is not known whether this gene is essential for virus replication.

The DNA sequence located upstream of the VV 01L-like ORF (see Figure 2A) would be expected to contain a swinepox viral promoter. This swinepox viral promoter will be useful as the control element of foreign DNA introduced into the swinepox genome.

Example 2

S-SPV-003

S-SPV-003 is a swinepox virus that expresses a foreign gene. The gene for *E.coli* β -galactosidase (lacZ gene) was inserted into the SPV 515-85.1 ORF. The foreign gene (lacZ) is under the control of a synthetic early/late promoter (EP1LP2).

S-SPV-003 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 520-17.5 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-003. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue

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indicating that the virus was pure, stable and expressing the foreign gene. The assays described here were carried out in VERO cells as well as EMSK cells, indicating that VERO cells would be a suitable substrate for the production of SPV recombinant vaccines. S-SPV-003 has been deposited with the ATCC under Accession No. VR 2335.

Example 3

10 S-SPV-008

S-SPV-008 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ gene) and the gene for pseudorabies virus (PRV) gp50 (26) were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a synthetic late promoter (LP1) and the gp50 gene is under the control of a synthetic early/late promoter (EP1LP2).

20 S-SPV-008 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.16 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-008. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

35

S-SPV-008 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Swine anti-PRV serum was

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shown to react specifically with S-SPV-008 plaques and not with S-SPV-009 negative control plaques. All S-SPV-008 observed plaques reacted with the swine antiserum indicating that the virus was stably expressing the PRV foreign gene. The black plaque assay was also performed on unfixed monolayers. The SPV plaques on the unfixed monolayers also exhibited specific reactivity with swine anti-PRV serum indicating that the PRV antigen is expressed on the infected cell surface.

To confirm the expression of the PRV gp50 gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. The swine anti-PRV serum was used to detect expression of PRV specific proteins. As shown in figure 6, the lysate from S-SPV-008 infected cells exhibits a specific band of approximately 48 kd, the reported size of PRV gp50 (35).

PRV gp50 is the gD homologue of HSV-1 (26). Several investigators have shown that VV expressing HSV-1 gD will protect mice against challenge with HSV-1 (6 and 34). Therefore the S-SPV-008 should be valuable as a vaccine to protect swine against PRV disease.

It is anticipated that several other PRV glycoproteins will be useful in the creation of recombinant swinepox vaccines to protect against PRV disease. These PRV glycoproteins include gpII (28), gpIII (27), and gpH (19). The PRV gpIII coding region has been engineered behind several synthetic pox promoters. The techniques utilized for the creation of S-SPV-008 will be used to create recombinant swinepox viruses expressing all four of these PRV glycoprotein genes. Such recombinant swinepox viruses will be useful as vaccines against PRV disease. Since the PRV vaccines described here do not express PRV gpX or gpI, they would be compatible with

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current PRV diagnostic tests (gX HerdChek®, gI HerdChek® and ClinEase®) which are utilized to distinguish vaccinated animals from infected animals. S-SPV-008 has been deposited with the ATCC under Accession No. VR 2339.

5

Example 4S-SPV-009

10 S-SPV-009 is a swinepox virus that expresses at least two foreign genes. The gene for *E. coli* β -galactosidase (lacZ gene) and the gene for Newcastle's Disease virus hemagglutinin (HN) gene were inserted into the SPV 515-85.1 ORF. The lacZ gene is under the control of a
15 synthetic late promoter (LP1) and the HN gene is under the control of an synthetic early/late promoter (EP1LP2).

20 S-SPV-009 was derived from S-SPV-001 (Kasza strain). This was accomplished utilizing the homology vector 538-46.26 (see Materials and Methods) and virus S-SPV-001 in the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV. The transfection stock was screened by the SCREEN FOR RECOMBINANT SPV EXPRESSING β -GALACTOSIDASE
25 (BLUOGAL AND CPRG ASSAYS). The final result of red plaque purification was the recombinant virus designated S-SPV-009. This virus was assayed for β -galactosidase expression, purity, and insert stability by multiple passages monitored by the blue plaque assay as described
30 in Materials and Methods. After the initial three rounds of purification, all plaques observed were blue indicating that the virus was pure, stable and expressing the marker gene.

35 S-SPV-009 was assayed for expression of PRV specific antigens using the BLACK PLAQUE SCREEN FOR FOREIGN GENE EXPRESSION IN RECOMBINANT SPV. Rabbit anti-NDV HN serum

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was shown to react specifically with S-SPV-009 plaques and not with S-SPV-008 negative control plaques. All S-SPV-009 observed plaques reacted with the swine antiserum indicating that the virus was stably expressing the NDV foreign gene. S-SPV-009 has been deposited with the ATCC under Acession No. VR 2344).

To confirm the expression of the NDV HN gene product, cells were infected with SPV and samples of infected cell lysates were subjected to SDS-polyacrylamide gel electrophoresis. The gel was blotted and analyzed using the WESTERN BLOTTING PROCEDURE. The rabbit anti-NDV HN serum was used to detect expression of the HN protein. The lysate from S-SPV-009 infected cells exhibited a specific band of approximately 74 kd, the reported size of NDV HN (29).

Example 5

Homology Vector 520-90.15

The homology vector 520-90.15 is a plasmid useful for the insertion of foreign DNA into SPV. Plasmid 520-90.15 contains a unique NdeI restriction site into which foreign DNA may be cloned. A plasmid containing such a foreign DNA insert has been used according to the HOMOLOGOUS RECOMBINATION PROCEDURE FOR GENERATING RECOMBINANT SPV to generate a SPV containing the foreign DNA. For this procedure to be successful, it is important that the insertion site be in a region non-essential to the replication of the SPV and that the site be flanked with swinepox virus DNA appropriate for mediating homologous recombination between virus and plasmid DNAs. The unique NdeI restriction site in plasmid 520-90.15 is located within the coding region of the SPV thymidine kinase gene (32). Therefore, we have shown that th thymidine kinase gene of swinepox virus is non-essential for DNA replication and is an appropriate insertion site.

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Example 6

5 The development of vaccines utilizing the swinepox virus to express antigens from various disease causing microorganisms can be engineered.

TRANSMISSIBLE GASTROENTERITIS VIRUS

10 The major neutralizing antigen of the transmissible gastroenteritis virus (TGE), glycoprotein 195, for use in the swinepox virus vector has been cloned. The clone of the neutralizing antigen is disclosed in U.S. Serial No. 078,519, filed July 27, 1987. It is contemplated that the procedures that have been used to express PRV gp50 in SPV and are disclosed herein are applicable to TGE.

15

PORCINE PARVOVIRUS

20 We have cloned the major capsid protein of the porcine (swine) parvovirus (PPV) for use in the swinepox virus vector. The clone of the capsid protein is disclosed in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV gp50 in SPV and are disclosed herein are applicable to PPV.

25 SWINE ROTAVIRUS

We have cloned the major neutralizing antigen of the swine rotavirus, glycoprotein 38, for use in the swinepox virus vector. The clone of glycoprotein 38 is disclosed in U.S. Patent No. 5,068,192 issued November 26, 1991. It is contemplated that the procedures that have been used to express PRV gp50 in SPV and are disclosed herein are applicable to SRV.

30

HOG CHOLERA VIRUS

35 The major neutralizing antigen of the bovine viral diarrhea (BVD) virus was cloned as disclosed in U.S. Serial No. 225,032, filed July 27, 1988. Since the BVD and hog cholera viruses are cross protective (31), the

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BVD virus antigen has been targeted for use in the swinepox virus vector. It is contemplated that the procedures that have been used to express PRV gp50 in SPV and are disclosed herein are applicable to BVD virus.

5

SERPULINA HYODYSENTERIAE

A protective antigen of *Serpulina hyodysenteriae* (3), for use in the swinepox virus vector has been cloned. It is contemplated that the procedures that have been used to express PRV gp50 in SPV and are disclosed herein are also applicable to *Serpulina hyodysenteriae*.

10

Antigens from the following microorganisms may also be utilized to develop animal vaccines: Swine influenza virus, foot and mouth disease virus, African swine fever virus, hog cholera virus and *Mycoplasma hyodysenteriae*.

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References

1. C. Bertholet, et al., *EMBO Journal* 5, 1951-1957 (1986).
2. R. A. Bhat, et al., *Nucleic Acids Research* 17, 1159-1176 (1989).
3. D. A. Boyden, et al., *Infection and Immunity* 57, 3808-3815 (1989).
4. D. B. Boyle and B. E. H. Coupar, *Virus Research* 10, 343-356 (1988).
5. R. M. Buller, et al., *Nature* 317, 813-815 (1985).
6. K. J. Cremer, et al., *Science* 228, 737-739 (1985).
7. A. J. Davidson and B. Moss, *J. Mol. Biol.* 210, 749-769 (1989).
8. A. J. Davidson and B. Moss, *J. Mol. Biol.* 210, 771-784 (1989).
9. P. L. Earl, et al., *Journal of Virology* 64, 2448-2451 (1990).
10. J. J. Esposito, et al., *Virology* 165, 313 (1988).
11. F. A. Ferrari, et al., *J. of Bacteriology* 161, 556-562 (1985).
12. C. Flexner, et al., *Vaccine* 8, 17-21 (1990).
13. S. J. Goebel, et al., *Virology* 179, 247-266 (1990).

SUBSTITUTE SHEET

-38-

14. U. Gubler and B. J. Hoffman, *Gene* **25**, 263-269 (1983).
15. M. A. Innis, et al., *PCR Protocols A Guide to Methods and Applications*, 84-91, Academic Press, Inc., San Diego (1990).
16. S. Joshi, et al., *Journal of Virology*. **65**, 5524-5530 (1991).
17. L. Kasza, et al., *Am. J. Vet. Res.* **21**, 269-273 (1960).
18. L. Kasza, *Diseases of Swine*, 254-260, Ed. A.D. Leman, et al., The Iowa State University Press, Ames, Iowa (1981).
19. B. G. Klupp and T. C. Mettenleiter, *Virology* **182**, 732-741 (1991).
20. U. K. Laemli, *Nature* **227**, 680-685 (1970).
21. B. Lominiczi, et al., *Journal of Virology* **49**, 970-979 (1984).
22. T. Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1982).
23. R. F. Massung, and R. W. Moyer, *Virology* **180**, 347-354 (1991).
24. R. F. Massung, and R. W. Moyer, *Virology* **180**, 355-364 (1991).
25. B. Moss, *Science* **252**, 1662-1667 (1991).

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26. E. A. Petrovskis, et al., *Journal of Virology* **59**, 216-223 (1986).
27. A. K. Robbins et al., *Journal of Virology* **58**, 339-347 (1986).
28. A. K. Robbins et al., *Journal of Virology* **61**, 2691-2701 (1987).
29. A. C. R. Samson, *Journal of Virology* **67**, 1199-1203 (1986).
30. J. Sambrook, et al., *Molecular Cloning A Laboratory Manual Second Edition*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989).
31. Sheffy, et al., *Proceedings 65th Annual Meeting of the United States Livestock Association* **65**, 347-353 (1961).
32. W. M. Schnitzlein and D. N. Tripathy, *Virology* **181**, 727-732, (1991).
33. J. Taylor, et al., *Vaccine* **9**, 190-193, (1991).
34. M. Wachsman, et al., *Journal of General Virology* **70**, 2513-2520 (1989).
35. M. W. Wathen, et al., *Journal of Virology* **51**, 57-62 (1984).
36. M. Weerasinge, *Journal of Virology* **65**, 5531-5534 (1991).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cochran Ph.D., Mark D
Junker M.S., David E
- (ii) TITLE OF INVENTION: Recombinant Swinepox Virus
- (iii) NUMBER OF SEQUENCES: 42
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: John P. White
 - (B) STREET: 30 Rockefeller Plaza
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: USA
 - (F) ZIP: 10112
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: White, John P
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212)977-9550
 - (B) TELEFAX: (212)664-0525
 - (C) TELEX: 422523

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 599 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Swinepox virus
 - (B) STRAIN: Kasza
 - (C) INDIVIDUAL ISOLATE: S-SPV-001
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: 515-85.1
- (viii) POSITION IN GENOME:
 - (B) MAP POSITION: -23.2
 - (C) UNITS: %G

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(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 202..597
- (D) OTHER INFORMATION: /partial
/codon_start= 202
/function= "Potential eukaryotic transcriptional
regulatory protein"
/standard_name= "515-85.1 ORF"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AATGTATCCA GAGTTGTTGA ATGCCTTATC GTACCTAATA TTAATATAGA GTTATTAAC 60
GAATAAGTAT ATATAAATGA TTGTTTTTAT AATGTTTGTG ATCGCATTGA GTTTTGCTGT 120
ATGGTTATCA TATACATTTT TAAGGCCGTA TATGATAAAT GAAAATATAT AAGCACTTAT 180
TTTTGTTAGT ATAATAACAC A ATG CCG TCG TAT ATG TAT CCG AAG AAC GCA 231
                        Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala
                        1           5           10
AGA AAA GTA ATT TCA AAG ATT ATA TCA TTA CAA CTT GAT ATT AAA AAA 279
Arg Lys Val Ile Ser Lys Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys
                        15           20           25
CTT CCT AAA AAA TAT ATA AAT ACC ATG TTA GAA TTT GGT CTA CAT GGA 327
Leu Pro Lys Lys Tyr Ile Asn Thr Met Leu Glu Phe Gly Leu His Gly
                        30           35           40
AAT CTA CCA GCT TGT ATG TAT AAA GAT GCC GTA TCA TAT GAT ATA AAT 375
Asn Leu Pro Ala Cys Met Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn
                        45           50           55
AAT ATA AGA TTT TTA CCT TAT AAT TGT GTT ATG GTT AAA GAT TTA ATA 423
Asn Ile Arg Phe Leu Pro Tyr Asn Cys Val Met Val Lys Asp Leu Ile
                        60           65           70
AAT GTT ATA AAA TCA TCA TCT GTA ATA GAT ACT AGA TTA CAT CAA TCT 471
Asn Val Ile Lys Ser Ser Ser Val Ile Asp Thr Arg Leu His Gln Ser
                        75           80           85           90
GTA TTA AAA CAT CGT AGA GCG TTA ATA GAT TAC GGC GAT CAA GAC ATT 519
Val Leu Lys His Arg Arg Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile
                        95           100           105
ATC ACT TTA ATG ATC ATT AAT AAG TTA CTA TCG ATA GAT GAT ATA TCC 567
Ile Thr Leu Met Ile Ile Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser
                        110           115           120
TAT ATA TTA GAT AAA AAA ATA ATT CAT GTA AC 599
Tyr Ile Leu Asp Lys Lys Ile Ile His Val
                        125           130

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg Lys Val Ile Ser Lys
 1           5           10           15
Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu Pro Lys Lys Tyr Ile
 20           25
Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn Leu Pro Ala Cys Met
 35           40           45
Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn Ile Arg Phe Leu Pro
 50           55           60
Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn Val Ile Lys Ser Ser
 65           70           75           80
Ser Val Ile Asp Thr Arg Leu His Gln Ser Val Leu Lys His Arg Arg
 85           90           95
Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile Thr Leu Met Ile Ile
 100          105          110
Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr Ile Leu Asp Lys Lys
 115          120          125
Ile Ile His Val
 130

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 899 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza
- (C) INDIVIDUAL ISOLATE: S-SPV-001

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 515-85.1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..662
- (D) OTHER INFORMATION: /partial
/codon_start= 3
/function= "Potential eukaryotic transcriptional
regulatory protein"
/standard_name= "515-85.1 ORF"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GA GAT ATT AAA TCA TGT AAA TGC TCG ATA TGT TCC GAC TCT ATA ACA Asp Ile Lys Ser Cys Lys Cys Ser Ile Cys Ser Asp Ser Ile Thr 1 5 10 15	47
CAT CAT ATA TAT GAA ACA ACA TCA TGT ATA AAT TAT AAA TCT ACC GAT His His Ile Tyr Glu Thr Thr Ser Cys Ile Asn Tyr Lys Ser Thr Asp 20 25 30	95
AAT GAT CTT ATG ATA GTA TTG TTC AAT CTA ACT AGA TAT TTA ATG CAT Asn Asp Leu Met Ile Val Leu Phe Asn Leu Thr Arg Tyr Leu Met His 35 40 45	143
GGG ATG ATA CAT CCT AAT CTT ATA AGC GTA AAA GGA TGG GGT CCC CTT Gly Met Ile His Pro Asn Leu Ile Ser Val Lys Gly Trp Gly Pro Leu 50 55 60	191
ATT GGA TTA TTA ACG GGT GAT ATA GGT ATT AAT TTA AAA CTA TAT TCC Ile Gly Leu Leu Thr Gly Asp Ile Gly Ile Asn Leu Lys Leu Tyr Ser 65 70 75	239
ACC ATG AAT ATA AAT GGG CTA CGG TAT GGA GAT ATT ACG TTA TCT TCA Thr Met Asn Ile Asn Gly Leu Arg Tyr Gly Asp Ile Thr Leu Ser Ser 80 85 90 95	287
TAC GAT ATG AGT AAT AAA TTA GTC TCT ATT ATT AAT ACA CCC ATA TAT Tyr Asp Met Ser Asn Lys Leu Val Ser Ile Ile Asn Thr Pro Ile Tyr 100 105 110	335
GAG TTA ATA CCG TTT ACT ACA TGT TGT TCA CTC AAT GAA TAT TAT TCA Glu Leu Ile Pro Phe Thr Thr Cys Cys Ser Leu Asn Glu Tyr Tyr Ser 115 120 125	383
AAA ATT GTG ATT TTA ATA AAT GTT ATT TTA GAA TAT ATG ATA TCT ATT Lys Ile Val Ile Leu Ile Asn Val Ile Leu Glu Tyr Met Ile Ser Ile 130 135 140	431
ATA TTA TAT AGA ATA TTG ATC GTA AAA AGA TTT AAT AAC ATT AAA GAA Ile Leu Tyr Arg Ile Leu Ile Val Lys Arg Phe Asn Asn Ile Lys Glu 145 150 155	479
TTT ATT TCA AAA GTC GTA AAT ACT GTA CTA GAA TCA TCA GGC ATA TAT Phe Ile Ser Lys Val Val Asn Thr Val Leu Glu Ser Ser Gly Ile Tyr 160 165 170 175	527
TTT TGT CAG ATG CGT GTA CAT GAA CAA ATT GAA TTG GAA ATA GAT GAG Phe Cys Gln Met Arg Val His Glu Gln Ile Glu Leu Glu Ile Asp Glu 180 185 190	575
CTC ATT ATT AAT GGA TCT ATG CCT GTA CAG CTT ATG CAT TTA CTT CTA Leu Ile Ile Asn Gly Ser Met Pro Val Gln Leu Met His Leu Leu Leu 195 200 205	623
AAG GTA GCT ACC ATA ATA TTA GAG GAA ATC AAA GAA ATA TAACGTATTT Lys Val Ala Thr Ile Ile Leu Glu Glu Ile Lys Glu Ile 210 215 220	672
TTTCTTTTAA ATAAATAAAA ATACTTTTTT TTTTAAACAA GGGGTGCTAC CTTGTCTAAT	732
TGTATCTTGT ATTTTGGATC TGATGCAAGA TTATTAAATA ATCGTATGAA AAAGTAGTAG	792
ATATAGTTTA TATCGTTACT GGACATGATA TTATGTTTAG TTAATTCCTC TTTGGCATGA	852
ATTCTACACG TCGGANAAGG TAATGTATCT ATAATGGTAT AAAGCTT	899

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 220 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Asp Ile Lys Ser Cys Lys Cys Ser Ile Cys Ser Asp Ser Ile Thr His
 1             5             10             15
His Ile Tyr Glu Thr Thr Ser Cys Ile Asn Tyr Lys Ser Thr Asp Asn
          20             25             30
Asp Leu Met Ile Val Leu Phe Asn Leu Thr Arg Tyr Leu Met His Gly
          35             40             45
Met Ile His Pro Asn Leu Ile Ser Val Lys Gly Trp Gly Pro Leu Ile
          50             55             60
Gly Leu Leu Thr Gly Asp Ile Gly Ile Asn Leu Lys Leu Tyr Ser Thr
 65             70             75             80
Met Asn Ile Asn Gly Leu Arg Tyr Gly Asp Ile Thr Leu Ser Ser Tyr
          85             90             95
Asp Met Ser Asn Lys Leu Val Ser Ile Ile Asn Thr Pro Ile Tyr Glu
          100            105            110
Leu Ile Pro Phe Thr Thr Cys Cys Ser Leu Asn Glu Tyr Tyr Ser Lys
          115            120            125
Ile Val Ile Leu Ile Asn Val Ile Leu Glu Tyr Met Ile Ser Ile Ile
          130            135            140
Leu Tyr Arg Ile Leu Ile Val Lys Arg Phe Asn Asn Ile Lys Glu Phe
          145            150            155            160
Ile Ser Lys Val Val Asn Thr Val Leu Glu Ser Ser Gly Ile Tyr Phe
          165            170            175
Cys Gln Met Arg Val His Glu Gln Ile Glu Leu Glu Ile Asp Glu Leu
          180            185            190
Ile Ile Asn Gly Ser Met Pro Val Gln Leu Met His Leu Leu Leu Lys
          195            200            205
Val Ala Thr Ile Ile Leu Glu Glu Ile Lys Glu Ile
          210            215            220

```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 129 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

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(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Vaccinia virus
 (B) STRAIN: Copenhagen

(viii) POSITION IN GENOME:
 (B) MAP POSITION: -23.2
 (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met	Phe	Met	Tyr	Pro	Glu	Phe	Ala	Arg	Lys	Ala	Leu	Ser	Lys	Leu	Ile
1				5					10					15	
Ser	Lys	Lys	Leu	Asn	Ile	Glu	Lys	Val	Ser	Ser	Lys	His	Gln	Leu	Val
			20					25					30		
Leu	Leu	Asp	Tyr	Gly	Leu	His	Gly	Leu	Leu	Pro	Lys	Ser	Leu	Tyr	Leu
		35					40					45			
Glu	Ala	Ile	Asn	Ser	Asp	Ile	Leu	Asn	Val	Arg	Phe	Phe	Pro	Pro	Glu
	50					55					60				
Ile	Ile	Asn	Val	Thr	Asp	Ile	Val	Lys	Ala	Leu	Gln	Asn	Ser	Cys	Arg
65				70					75					80	
Val	Asp	Glu	Tyr	Leu	Lys	Ala	Val	Ser	Leu	Tyr	His	Lys	Asn	Ser	Leu
			85						90					95	
Met	Val	Ser	Gly	Pro	Asn	Val	Val	Lys	Leu	Met	Ile	Glu	Tyr	Asn	Leu
			100					105					110		
Leu	Thr	His	Ser	Asp	Leu	Glu	Trp	Leu	Ile	Asn	Glu	Asn	Val	Val	Lys
		115					120					125			

Ala

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 132 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Swinepox virus

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(B) STRAIN: Kasza

(viii) POSITION IN GENOME:
 (B) MAP POSITION: -23.2
 (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Pro Ser Tyr Met Tyr Pro Lys Asn Ala Arg Lys Val Ile Ser Lys
1           5           10           15
Ile Ile Ser Leu Gln Leu Asp Ile Lys Lys Leu Pro Lys Lys Tyr Ile
20           25           30
Asn Thr Met Leu Glu Phe Gly Leu His Gly Asn Leu Pro Ala Cys Met
35           40           45
Tyr Lys Asp Ala Val Ser Tyr Asp Ile Asn Asn Ile Arg Phe Leu Pro
50           55           60
Tyr Asn Cys Val Met Val Lys Asp Leu Ile Asn Val Ile Lys Ser Ser
65           70           75           80
Ser Val Ile Asp Thr Arg Leu His Gln Ser Val Leu Lys His Arg Arg
85           90           95
Ala Leu Ile Asp Tyr Gly Asp Gln Asp Ile Ile Thr Leu Met Ile Ile
100          105          110
Asn Lys Leu Leu Ser Ile Asp Asp Ile Ser Tyr Ile Leu Asp Lys Lys
115          120          125
Ile Ile His Val
130

```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Vaccinia virus
 (B) STRAIN: Copenhagen

(viii) POSITION IN GENOME:

(B) MAP POSITION: -23.2
 (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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```

Val Leu Asn Asp Gln Tyr Ala Lys Ile Val Ile Phe Phe Asn Thr Ile
1           5           10           15
Ile Glu Tyr Ile Ile Ala Thr Ile Tyr Tyr Arg Leu Thr Val Leu Asn
20          25          30
Asn Tyr Thr Asn Val Lys His Phe Val Ser Lys Val Leu His Thr Val
35          40          45
Met Glu Ala Cys Gly Val Leu Phe Ser Tyr Ile Lys Val Asn Asp Lys
50          55          60
Ile Glu His Glu Leu Glu Glu Met Val Asp Lys Gly Thr Val Pro Ser
65          70          75          80
Tyr Leu Tyr His Leu Ser Ile Asn Val Ile Ser Ile Ile Leu Asp Asp
85          90          95
Ile Asn Gly Thr Arg
100

```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Swinepox virus
- (B) STRAIN: Kasza

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -23.2
- (C) UNITS: %G

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Ser Leu Asn Glu Tyr Tyr Ser Lys Ile Val Ile Leu Ile Asn Val Ile
1           5           10           15
Leu Glu Tyr Met Ile Ser Ile Ile Leu Tyr Arg Ile Leu Ile Val Lys
20          25          30
Arg Phe Asn Asn Ile Lys Glu Phe Ile Ser Lys Val Val Asn Thr Val
35          40          45
Leu Glu Ser Ser Gly Ile Tyr Phe Cys Gln Met Arg Val His Glu Gln
50          55          60
Ile Glu Leu Glu Ile Asp Glu Leu Ile Ile Asn Gly Ser Met Pro Val
65          70          75          80
Gln Leu Met His Leu Leu Leu Lys Val Ala Thr Ile Ile Leu Glu Glu

```

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85

90

95

Ile Lys Glu Ile
100

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 520-17.5 (Junction A)

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Ferrari, Franco A
Trach, Kathleen
Hoch, James A

(B) TITLE: Sequence Analysis of the spoB Locus Reveals a
Polycistronic Transcription Unit

(C) JOURNAL: J. Bacteriol.

(D) VOLUME: 161

(E) ISSUE: 2

(F) PAGES: 556-562

(G) DATE: Feb.-1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CACATACGAT TTAGGTGACA CTATAGAATA CAAGCTTTAT ACCATTATAG ATACATTACC 60

TTGTCCGACG TGTA GAATTC ATGCCAAAGA AGAATTA ACT AA 102

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 520-17.5 (Junction B)

(ix) FEATURE:

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(A) NAME/KEY: CDS
 (B) LOCATION: 85..99
 (D) OTHER INFORMATION: /codon_start= 85
 /function= "Translational start of hybrid protein"
 /product= "N-terminal peptide"
 /number= 1
 /standard_name= "Translation of synthetic DNA
 sequence"

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 100..102
 (C) IDENTIFICATION METHOD: experimental
 (D) OTHER INFORMATION: /partial
 /codon_start= 100
 /function= "marker enzyme"
 /product= "Beta-Galactosidase"
 /evidence= EXPERIMENTAL
 /gene= "lacZ"
 /number= 2
 /citation= ([1])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Ferrari, Franco A
 Trach, Kathleen
 Hoch, James A
 (B) TITLE: Sequence Analysis of the spoOB Locus Reveals
 a Polycistronic Transcription Unit
 (C) JOURNAL: J. Bacteriol.
 (D) VOLUME: 161
 (E) ISSUE: 2
 (F) PAGES: 556-562
 (G) DATE: Feb.-1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTAGTCGACT CTAGAAAAAA TTGAAAAACT ATTCTAATTT ATTGCACGGA GATCTTTTTT	60
TTTTTTTTTT TTTTGGCAT ATAA ATG AAT TCG GAT CCC GTC	102
Met Asn Ser Asp Pro Val	
1 5 1	

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Asn Ser Asp Pro
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Val
1

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 103 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 520-17.5 (Junction C)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..72
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /partial
 - /codon start= 1
 - /function= "marker enzyme"
 - /product= "Beta-galactosidase"
 - /evidence= EXPERIMENTAL
 - /gene= "lacZ"
 - /number= 1
 - /citation= ([1])

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 73..78
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /codon start= 73
 - /function= "Translational finish of hybrid protein"
 - /product= "C-terminal peptide"
 - /evidence= EXPERIMENTAL
 - /number= 2
 - /standard name= "Translation of synthetic DNA sequence"

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Ferrari, Franco A
Trach, Kathleen
Hoch, James A
- (B) TITLE: Sequence Analysis of the spo0B Locus Reveals
a Polycistronic Transcription Unit
- (C) JOURNAL: J. Bacteriol.
- (D) VOLUME: 161
- (E) ISSUE: 2
- (F) PAGES: 556-562
- (G) DATE: Feb.-1985

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGC	CCG	TCA	GTA	TCG	GCG	GAA	ATC	CAG	CTG	AGC	GCC	GGT	CGC	TAC	CAT	48
Ser	Pro	Ser	Val	Ser	Ala	Glu	Ile	Gln	Leu	Ser	Ala	Gly	Arg	Tyr	His	
1				5					10					15		
TAC	CAG	TTG	GTC	TGG	TGT	CAA	AAA	GAT	CCA	TAATTAATTA	ACCCGGGTCG	98				
Tyr	Gln	Leu	Val	Trp	Cys	Gln	Lys	Asp	Pro							
			20					1								
AAGAC											103					

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ser	Pro	Ser	Val	Ser	Ala	Glu	Ile	Gln	Leu	Ser	Ala	Gly	Arg	Tyr	His
1				5					10					15	
Tyr	Gln	Leu	Val	Trp	Cys	Gln	Lys								
			20												

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp	Pro
1	

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 520-17.5 (Junction D)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGATCCCCGG GCGAGCTCGA ATTCGTAATC ATGGTCATAG CTGTTTCC

48

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Plasmid

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: 538-46.26 (Junction A)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CACATACGAT TTAGGTGACA CTATAGAATA CAAGCTTTAT ACCATTATAG ATACATT

57

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Plasmid

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: 538-46.16 (Junction B)

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 91..102
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /partial
 - /codon_start= 91
 - /function= "marker enzyme"
 - /product= "Beta-Galactosidase"
 - /evidence= EXPERIMENTAL
 - /gene= "lacZ"
 - /number= 2
 - /citation= {[1]}

(ix) FEATURE:

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(A) NAME/KEY: CDS
 (B) LOCATION: 76..90
 (D) OTHER INFORMATION: /partial
 /codon_start= 76
 /function= "Translational start of hybrid protein"
 /product= "N-terminal peptide"
 /number= 1
 /standard_name= "Translation of synthetic DNA
 sequence"

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Ferrari, Franco A
 Trach, Kathleen
 Hoch, James A
 (B) TITLE: Sequence Analysis of the spoOB Locus Reveals
 a Polycistronic Transcription Unit
 (C) JOURNAL: J. Bacteriol.
 (D) VOLUME: 161
 (E) ISSUE: 2
 (F) PAGES: 556-562
 (G) DATE: Feb.-1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
AAGCTGGTAG ATTTCCATGT AGGGCCGCCT GCAGGTCGAC TCTAGAATTT CATTTTGTTT      60
TTTTCTATGC TATAA ATG AAT TCG GAT CCC GTC GTT TTA CAA      102
          Met Asn Ser Asp Pro Val Val Leu Gln
                1             5     1
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Met Asn Ser Asp Pro
 1             5
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Val Val Leu Gln
 1
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 206 base pairs
 (B) TYPE: nucleic acid

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- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Plasmid
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: 538-46.16 (Junction C)
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..63
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /partial
 - /codon_start= 1
 - /function= "marker enzyme"
 - /product= "Beta-galactosidase"
 - /evidence= EXPERIMENTAL
 - /number= 1
 - /citation= ([1])
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 64..69
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /codon_start= 64
 - /function= "Translational finish of hybrid protein"
 - /product= "C-terminal peptide"
 - /evidence= EXPERIMENTAL
 - /standard_name= "Translation of synthetic DNA sequence"
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 177..185
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /codon_start= 177
 - /function= "Translational start of hybrid protein"
 - /product= "N-terminal peptide"
 - /evidence= EXPERIMENTAL
 - /standard_name= "Translation of synthetic DNA sequence"
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 186..206
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /partial
 - /codon_start= 186
 - /function= "glycoprotein"
 - /product= "PRV gp50"
 - /evidence= EXPERIMENTAL
 - /gene= "gp50"
 - /number= 3
 - /citation= ([2])
- (x) PUBLICATION INFORMATION:
 - (A) AUTHORS: Ferrari, Franco A

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Trach, Kathleen

Hoch, James A

(B) TITLE: Sequence Analysis of the spoOB Locus Reveals
a Polycistronic Transcription Unit

(C) JOURNAL: J. Bacteriol.

(D) VOLUME: 161

(E) ISSUE: 2

(F) PAGES: 556-562

(G) DATE: Feb.-1985

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Petrovskis, Erik A

Timmins, James G

Armentrout, Marty A

Marchioli, Carmine C

Jr. Yancy, Robert J

Post, Leonard E

(B) TITLE: DNA Sequence of the Gene for Pseudorabies
Virus gp50, a Glycoprotein without N-Linked
Glycosylation

(C) JOURNAL: J. Virol.

(D) VOLUME: 59

(E) ISSUE: 2

(F) PAGES: 216-223

(G) DATE: Aug.-1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTA TCG GCG GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG	48
Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu	
1 5 10 15	
GTC TGG TGT CAA AAA GAT CCA TAATTAATTA ACCCGGCCGC CTGCAGGTGC	99
Val Trp Cys Gln Lys Asp Pro	
20 1	
ACTCTAGAAA AAATTGAAAA ACTATTCTAA TTTATTGCAC GGAGATCTTT TTTTTTTTTT	159
TTTTTTTGG CATATAA ATG AAT TCG CTC GCA GCG CTA TTG GCG GCG	206
Met Asn Ser Leu Ala Ala Leu Leu Ala Ala	
1 1 5	

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu	
1 5 10 15	
Val Trp Cys Gln Lys	
20	

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2 amino acids

(B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Asp Pro

1

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Asn Ser

1

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Leu Ala Ala Leu Leu Ala Ala

1

5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 101 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 538-46.16 (Junction D)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..15

(D) OTHER INFORMATION: /partial

/codon_start= 1

/function= "glycoprotein"

/product= "PRV gp63"

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/gene= "gp63"
 /number= 1
 /citation= ([1])

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Petrovskis, Erik A
 Timmins, James G
 Post, Lenoard E

(B) TITLE: Use of Lambda-gt11 To Isolate Genes for two
 Pseudorabies Virus Glycoproteins with homology to
 Herpes Simplex Virus and Varicella-Zoster Virus
 Glycoproteins

(C) JOURNAL: J. Virol.

(D) VOLUME: 60

(E) ISSUE: 1

(F) PAGES: 185-193

(G) DATE: Oct.-1986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGC GTG CAC CAC GAG GGACTCTAGA GGATCCATAA TTAATTAATT AATTTTATC 55
 Arg Val His His Glu
 1 5

CCGGGTCGAC CTGCAGGCGG CCGGGTCGAC CTGCAGGCGG CCAGAC 101

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Arg Val His His Glu
 1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 538-46.16 (Junction E)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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AGATCCCCGG GCGAGCTCGA ATTCGTAATC ATGGTCATAG CTGTTTCCTG TGTGAAA

57

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1907 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Newcastle disease virus
- (B) STRAIN: B1

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 137-23.803 (PSY1142)

(viii) POSITION IN GENOME:

- (B) MAP POSITION: -50%
- (C) UNITS: %G

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 92..1822
- (D) OTHER INFORMATION: /codon_start= 92
/product= "NDV heamagglutinin-Neuraminidase"
/gene= "HN"
/number= 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACGGGTAGAA CGGTAAGAGA GGCCGCCCCT CAATTGCGAG CCAGACTTCA CAACCTCCGT	60
TCTACCGCTT CACCGACAAC AGTCCTCAAT C ATG GAC CGC GCC GTT AGC CAA	112
Met Asp Arg Ala Val Ser Gln	
1 5	
GTT GCG TTA GAG AAT GAT GAA AGA GAG GCA AAA AAT ACA TGG CGC TTG	160
Val Ala Leu Glu Asn Asp Glu Arg Glu Ala Lys Asn Thr Trp Arg Leu	
10 15 20	
ATA TTC CGG ATT GCA ATC TTA TTC TTA ACA GTA GTG ACC TTG GCT ATA	208
Ile Phe Arg Ile Ala Ile Leu Phe Leu Thr Val Val Thr Leu Ala Ile	
25 30 35	
TCT GTA GCC TCC CTT TTA TAT AGC ATG GGG GCT AGC ACA CCT AGC GAT	256
Ser Val Ala Ser Leu Leu Tyr Ser Met Gly Ala Ser Thr Pro Ser Asp	
40 45 50 55	
CTT GTA GGC ATA CCG ACT AGG ATT TCC AGG GCA GAA GAA AAG ATT ACA	304
Leu Val Gly Ile Pro Thr Arg Ile Ser Arg Ala Glu Glu Lys Ile Thr	
60 65 70	
TCT ACA CTT GGT TCC AAT CAA GAT GTA GTA GAT AGG ATA TAT AAG CAA	352
Ser Thr Leu Gly Ser Asn Gln Asp Val Val Asp Arg Ile Tyr Lys Gln	
75 80 85	
GTG GCC CTT GAG TCT CCA TTG GCA TTG TTA AAT ACT GAG ACC ACA ATT	400

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Val	Ala	Leu	Glu	Ser	Pro	Leu	Ala	Leu	Leu	Asn	Thr	Glu	Thr	Thr	Ile	
	90						95					100				
ATG	AAC	GCA	ATA	ACA	TCT	CTC	TCT	TAT	CAG	ATT	AAT	GGA	GCT	GCA	AAC	448
Met	Asn	Ala	Ile	Thr	Ser	Leu	Ser	Tyr	Gln	Ile	Asn	Gly	Ala	Ala	Asn	
	105					110					115					
AAC	AGC	GGG	TGG	GGG	GCA	CCT	ATT	CAT	GAC	CCA	GAT	TAT	ATA	GGG	GGG	496
Asn	Ser	Gly	Trp	Gly	Ala	Pro	Ile	His	Asp	Pro	Asp	Tyr	Ile	Gly	Gly	
	120				125				130					135		
ATA	GGC	AAA	GAA	CTC	ATT	GTA	GAT	GAT	GCT	AGT	GAT	GTC	ACA	TCA	TTC	544
Ile	Gly	Lys	Glu	Leu	Ile	Val	Asp	Asp	Ala	Ser	Asp	Val	Thr	Ser	Phe	
				140					145					150		
TAT	CCC	TCT	GCA	TTT	CAA	GAA	CAT	CTG	AAT	TTT	ATC	CCG	GCG	CCT	ACT	592
Tyr	Pro	Ser	Ala	Phe	Gln	Glu	His	Leu	Asn	Phe	Ile	Pro	Ala	Pro	Thr	
			155					160					165			
ACA	GGA	TCA	GGT	TGC	ACT	CGA	ATA	CCC	TCA	TTT	GAC	ATG	AGT	GCT	ACC	640
Thr	Gly	Ser	Gly	Cys	Thr	Arg	Ile	Pro	Ser	Phe	Asp	Met	Ser	Ala	Thr	
		170					175					180				
CAT	TAC	TGC	TAC	ACC	CAT	AAT	GTA	ATA	TTG	TCT	GGA	TGC	AGA	GAT	CAC	688
His	Tyr	Cys	Tyr	Thr	His	Asn	Val	Ile	Leu	Ser	Gly	Cys	Arg	Asp	His	
	185					190					195					
TCA	CAC	TCA	CAT	CAG	TAT	TTA	GCA	CTT	GGT	GTG	CTC	CGG	ACA	TCT	GCA	736
Ser	His	Ser	His	Gln	Tyr	Leu	Ala	Leu	Gly	Val	Leu	Arg	Thr	Ser	Ala	
	200				205				210						215	
ACA	GGG	AGG	GTA	TTC	TTT	TCT	ACT	CTG	CGT	TCC	ATC	AAC	CTG	GAC	GAC	784
Thr	Gly	Arg	Val	Phe	Phe	Ser	Thr	Leu	Arg	Ser	Ile	Asn	Leu	Asp	Asp	
			220						225					230		
ACC	CAA	AAT	CGG	AAG	TCT	TGC	AGT	GTG	AGT	GCA	ACT	CCC	CTG	GGT	TGT	832
Thr	Gln	Asn	Arg	Lys	Ser	Cys	Ser	Val	Ser	Ala	Thr	Pro	Leu	Gly	Cys	
			235					240					245			
GAT	ATG	CTG	TGC	TCG	AAA	GCC	ACG	GAG	ACA	GAG	GAA	GAA	GAT	TAT	AAC	880
Asp	Met	Leu	Cys	Ser	Lys	Ala	Thr	Glu	Thr	Glu	Glu	Glu	Asp	Tyr	Asn	
	250					255					260					
TCA	GCT	GTC	CCT	ACG	CGG	ATG	GTA	CAT	GGG	AGG	TTA	GGG	TTC	GAC	GGC	928
Ser	Ala	Val	Pro	Thr	Arg	Met	Val	His	Gly	Arg	Leu	Gly	Phe	Asp	Gly	
	265					270					275					
CAA	TAT	CAC	GAA	AAG	GAC	CTA	GAT	GTC	ACA	ACA	TTA	TTC	GGG	GAC	TGG	976
Gln	Tyr	His	Glu	Lys	Asp	Leu	Asp	Val	Thr	Thr	Leu	Phe	Gly	Asp	Trp	
	280				285					290					295	
GTG	GCC	AAC	TAC	CCA	GGA	GTA	GGG	GGT	GGA	TCT	TTT	ATT	GAC	AGC	CGC	1024
Val	Ala	Asn	Tyr	Pro	Gly	Val	Gly	Gly	Gly	Ser	Phe	Ile	Asp	Ser	Arg	
				300					305					310		
GTG	TGG	TTC	TCA	GTC	TAC	GGA	GGG	TTA	AAA	CCC	AAT	ACA	CCC	AGT	GAC	1072
Val	Trp	Phe	Ser	Val	Tyr	Gly	Gly	Leu	Lys	Pro	Asn	Thr	Pro	S	r	Asp
			315					320					325			
ACT	GTA	CAG	GAA	GGG	AAA	TAT	GTG	ATA	TAC	AAG	CGA	TAC	AAT	GAC	ACA	1120
Thr	Val	Gln	Glu	Gly	Lys	Tyr	Val	Ile	Tyr	Lys	Arg	Tyr	Asn	Asp	Thr	
	330						335					340				
TGC	CCA	GAT	GAG	CAA	GAC	TAC	CAG	ATT	CGA	ATG	GCC	AAG	TCT	TCG	TAT	1168

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Cys	Pro	Asp	Glu	Gln	Asp	Tyr	Gln	Ile	Arg	Met	Ala	Lys	Ser	Ser	Tyr	
345						350					355					
AAG	CCT	GGA	CGG	TTT	GGT	GGG	AAA	CGC	ATA	CAG	CAG	GCT	ATC	TTA	TCT	1216
Lys	Pro	Gly	Arg	Phe	Gly	Gly	Lys	Arg	Ile	Gln	Gln	Ala	Ile	Leu	Ser	
360					365					370					375	
ATC	AAA	GTG	TCA	ACA	TCC	TTA	GGC	GAA	GAC	CCG	GTA	CTG	ACT	GTA	CCG	1264
Ile	Lys	Val	Ser	Thr	Ser	Leu	Gly	Glu	Asp	Pro	Val	Leu	Thr	Val	Pro	
				380					385					390		
CCC	AAC	ACA	GTC	ACA	CTC	ATG	GGG	GCC	GAA	GGC	AGA	ATT	CTC	ACA	GTA	1312
Pro	Asn	Thr	Val	Thr	Leu	Met	Gly	Ala	Glu	Gly	Arg	Ile	Leu	Thr	Val	
			395					400					405			
GGG	ACA	TCC	CAT	TTC	TTG	TAT	CAG	CGA	GGG	TCA	TCA	TAC	TTC	TCT	CCC	1360
Gly	Thr	Ser	His	Phe	Leu	Tyr	Gln	Arg	Gly	Ser	Ser	Tyr	Phe	Ser	Pro	
		410					415					420				
GCG	TTA	TTA	TAT	CCT	ATG	ACA	GTC	AGC	AAC	AAA	ACA	GCC	ACT	CTT	CAT	1408
Ala	Leu	Leu	Tyr	Pro	Met	Thr	Val	Ser	Asn	Lys	Thr	Ala	Thr	Leu	His	
			425			430						435				
AGT	CCT	TAT	ACA	TTC	AAT	GCC	TTC	ACT	CGG	CCA	GGT	AGT	ATC	CCT	TGC	1456
Ser	Pro	Tyr	Thr	Phe	Asn	Ala	Phe	Thr	Arg	Pro	Gly	Ser	Ile	Pro	Cys	
440					445					450					455	
CAG	GCT	TCA	GCA	AGA	TGC	CCC	AAC	TCA	TGT	GTT	ACT	GGA	GTC	TAT	ACA	1504
Gln	Ala	Ser	Ala	Arg	Cys	Pro	Asn	Ser	Cys	Val	Thr	Gly	Val	Tyr	Thr	
				460					465					470		
GAT	CCA	TAT	CCC	CTA	ATC	TTC	TAT	AGA	AAC	CAC	ACC	TTG	CGA	GGG	GTA	1552
Asp	Pro	Tyr	Pro	Leu	Ile	Phe	Tyr	Arg	Asn	His	Thr	Leu	Arg	Gly	Val	
			475					480					485			
TTC	GGG	ACA	ATG	CTT	GAT	GGT	GAA	CAA	GCA	AGA	CTT	AAC	CCT	GCG	TCT	1600
Phe	Gly	Thr	Met	Leu	Asp	Gly	Glu	Gln	Ala	Arg	Leu	Asn	Pro	Ala	Ser	
		490				495						500				
GCA	GTA	TTC	GAT	AGC	ACA	TCC	CGC	AGT	CGC	ATA	ACT	CGA	GTG	AGT	TCA	1648
Ala	Val	Phe	Asp	Ser	Thr	Ser	Arg	Ser	Arg	Ile	Thr	Arg	Val	Ser	Ser	
			505			510						515				
AGC	AGC	ATC	AAA	GCA	GCA	TAC	ACA	ACA	TCA	ACT	TGT	TTT	AAA	GTG	GTC	1696
Ser	Ser	Ile	Lys	Ala	Ala	Tyr	Thr	Thr	Ser	Thr	Cys	Phe	Lys	Val	Val	
520					525					530					535	
AAG	ACC	AAT	AAG	ACC	TAT	TGT	CTC	AGC	ATT	GCT	GAA	ATA	TCT	AAT	ACT	1744
Lys	Thr	Asn	Lys	Thr	Tyr	Cys	Leu	Ser	Ile	Ala	Glu	Ile	Ser	Asn	Thr	
				540					545					550		
CTC	TTC	GGA	GAA	TTC	AGA	ATC	GTC	CCG	TTA	CTA	GTT	GAG	ATC	CTC	AAA	1792
Leu	Phe	Gly	Glu	Phe	Arg	Ile	Val	Pro	Leu	Leu	Val	Glu	Ile	Leu	Lys	
			555					560					565			
GAT	GAC	GGG	GTT	AGA	GAA	GCC	AGG	TCT	GGC	TAGTTGAGTC	AACTATGAAA					1842
Asp	Asp	Gly	Val	Arg	Glu	Ala	Arg	Ser	Gly							
			570				575									
GAGTTGGAAA	GATGGCATTG	TATCACCTAT	CTTCTGCGAC	ATCAAGAATC	AAACCGAATG											1902
CCGGC																1907

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(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 577 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```

Met Asp Arg Ala Val Ser Gln Val Ala Leu Glu Asn Asp Glu Arg Glu
 1           5           10           15
Ala Lys Asn Thr Trp Arg Leu Ile Phe Arg Ile Ala Ile Leu Phe Leu
          20           25           30
Thr Val Val Thr Leu Ala Ile Ser Val Ala Ser Leu Leu Tyr Ser Met
          35           40           45
Gly Ala Ser Thr Pro Ser Asp Leu Val Gly Ile Pro Thr Arg Ile Ser
          50           55           60
Arg Ala Glu Glu Lys Ile Thr Ser Thr Leu Gly Ser Asn Gln Asp Val
          65           70           75           80
Val Asp Arg Ile Tyr Lys Gln Val Ala Leu Glu Ser Pro Leu Ala Leu
          85           90           95
Leu Asn Thr Glu Thr Thr Ile Met Asn Ala Ile Thr Ser Leu Ser Tyr
          100          105          110
Gln Ile Asn Gly Ala Ala Asn Asn Ser Gly Trp Gly Ala Pro Ile His
          115          120          125
Asp Pro Asp Tyr Ile Gly Gly Ile Gly Lys Glu Leu Ile Val Asp Asp
          130          135          140
Ala Ser Asp Val Thr Ser Phe Tyr Pro Ser Ala Phe Gln Glu His Leu
          145          150          155          160
Asn Phe Ile Pro Ala Pro Thr Thr Gly Ser Gly Cys Thr Arg Ile Pro
          165          170          175
Ser Phe Asp Met Ser Ala Thr His Tyr Cys Tyr Thr His Asn Val Ile
          180          185          190
Leu Ser Gly Cys Arg Asp His Ser His Ser His Gln Tyr Leu Ala Leu
          195          200          205
Gly Val Leu Arg Thr Ser Ala Thr Gly Arg Val Phe Phe Ser Thr Leu
          210          215          220
Arg S r Ile Asn Leu Asp Asp Thr Gln Asn Arg Lys Ser Cys Ser Val
          225          230          235          240
Ser Ala Thr Pro Leu Gly Cys Asp Met Leu Cys Ser Lys Ala Thr Glu
          245          250          255
Thr Glu Glu Glu Asp Tyr Asn Ser Ala Val Pro Thr Arg Met Val His
          260          265          270
Gly Arg Leu Gly Phe Asp Gly Gln Tyr His Glu Lys Asp Leu Asp Val

```

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275										280										285																																					
Thr	Thr	Leu	Phe	Gly	Asp	Trp	Val	Ala	Asn	Tyr	Pro	Gly	Val	Gly	Gly					Thr	Thr	Leu	Phe	Gly	Asp	Trp	Val	Ala	Asn	Tyr	Pro	Gly	Val	Gly	Gly				Thr	Thr	Leu	Phe	Gly	Asp	Trp	Val	Ala	Asn	Tyr	Pro	Gly	Val	Gly	Gly			
		290																				295																		300																	
Gly	Ser	Phe	Ile	Asp	Ser	Arg	Val	Trp	Phe	Ser	Val	Tyr	Gly	Gly	Leu					Gly	Ser	Phe	Ile	Asp	Ser	Arg	Val	Trp	Phe	Ser	Val	Tyr	Gly	Gly	Leu			Gly	Ser	Phe	Ile	Asp	Ser	Arg	Val	Trp	Phe	Ser	Val	Tyr	Gly	Gly	Leu				
		305																				310																	315																		
Lys	Pro	Asn	Thr	Pro	Ser	Asp	Thr	Val	Gln	Glu	Gly	Lys	Tyr	Val	Ile					Lys	Pro	Asn	Thr	Pro	Ser	Asp	Thr	Val	Gln	Glu	Gly	Lys	Tyr	Val	Ile			Lys	Pro	Asn	Thr	Pro	Ser	Asp	Thr	Val	Gln	Glu	Gly	Lys	Tyr	Val	Ile				
				325																				325																330																	
Tyr	Lys	Arg	Tyr	Asn	Asp	Thr	Cys	Pro	Asp	Glu	Gln	Asp	Tyr	Gln	Ile					Tyr	Lys	Arg	Tyr	Asn	Asp	Thr	Cys	Pro	Asp	Glu	Gln	Asp	Tyr	Gln	Ile			Tyr	Lys	Arg	Tyr	Asn	Asp	Thr	Cys	Pro	Asp	Glu	Gln	Asp	Tyr	Gln	Ile				
				340																				340																345																	
Arg	Met	Ala	Lys	Ser	Ser	Tyr	Lys	Pro	Gly	Arg	Phe	Gly	Gly	Lys	Arg					Arg	Met	Ala	Lys	Ser	Ser	Tyr	Lys	Pro	Gly	Arg	Phe	Gly	Gly	Lys	Arg			Arg	Met	Ala	Lys	Ser	Ser	Tyr	Lys	Pro	Gly	Arg	Phe	Gly	Gly	Lys	Arg				
				355																				355																360																	
Ile	Gln	Gln	Ala	Ile	Leu	Ser	Ile	Lys	Val	Ser	Thr	Ser	Leu	Gly	Glu					Ile	Gln	Gln	Ala	Ile	Leu	Ser	Ile	Lys	Val	Ser	Thr	Ser	Leu	Gly	Glu			Ile	Gln	Gln	Ala	Ile	Leu	Ser	Ile	Lys	Val	Ser	Thr	Ser	Leu	Gly	Glu				
				370																				375																380																	
Asp	Pro	Val	Leu	Thr	Val	Pro	Pro	Asn	Thr	Val	Thr	Leu	Met	Gly	Ala					Asp	Pro	Val	Leu	Thr	Val	Pro	Pro	Asn	Thr	Val	Thr	Leu	Met	Gly	Ala			Asp	Pro	Val	Leu	Thr	Val	Pro	Pro	Asn	Thr	Val	Thr	Leu	Met	Gly	Ala				
					390																			395															400																		
Glu	Gly	Arg	Ile	Leu	Thr	Val	Gly	Thr	Ser	His	Phe	Leu	Tyr	Gln	Arg					Glu	Gly	Arg	Ile	Leu	Thr	Val	Gly	Thr	Ser	His	Phe	Leu	Tyr	Gln	Arg			Glu	Gly	Arg	Ile	Leu	Thr	Val	Gly	Thr	Ser	His	Phe	Leu	Tyr	Gln	Arg				
				405																				410															415																		
Gly	Ser	Ser	Tyr	Phe	Ser	Pro	Ala	Leu	Leu	Tyr	Pro	Met	Thr	Val	Ser					Gly	Ser	Ser	Tyr	Phe	Ser	Pro	Ala	Leu	Leu	Tyr	Pro	Met	Thr	Val	Ser			Gly	Ser	Ser	Tyr	Phe	Ser	Pro	Ala	Leu	Leu	Tyr	Pro	Met	Thr	Val	Ser				
				420																				425															430																		
Asn	Lys	Thr	Ala	Thr	Leu	His	Ser	Pro	Tyr	Thr	Phe	Asn	Ala	Phe	Thr					Asn	Lys	Thr	Ala	Thr	Leu	His	Ser	Pro	Tyr	Thr	Phe	Asn	Ala	Phe	Thr			Asn	Lys	Thr	Ala	Thr	Leu	His	Ser	Pro	Tyr	Thr	Phe	Asn	Ala	Phe	Thr				
				435																				440															445																		
Arg	Pro	Gly	Ser	Ile	Pro	Cys	Gln	Ala	Ser	Ala	Arg	Cys	Pro	Asn	Ser					Arg	Pro	Gly	Ser	Ile	Pro	Cys	Gln	Ala	Ser	Ala	Arg	Cys	Pro	Asn	Ser			Arg	Pro	Gly	Ser	Ile	Pro	Cys	Gln	Ala	Ser	Ala	Arg	Cys	Pro	Asn	Ser				
				450																				455															460																		
Cys	Val	Thr	Gly	Val	Tyr	Thr	Asp	Pro	Tyr	Pro	Leu	Ile	Phe	Tyr	Arg					Cys	Val	Thr	Gly	Val	Tyr	Thr	Asp	Pro	Tyr	Pro	Leu	Ile	Phe	Tyr	Arg			Cys	Val	Thr	Gly	Val	Tyr	Thr	Asp	Pro	Tyr	Pro	Leu	Ile	Phe	Tyr	Arg				
				465																				470															475																		
Asn	His	Thr	Leu	Arg	Gly	Val	Phe	Gly	Thr	Met	Leu	Asp	Gly	Glu	Gln					Asn	His	Thr	Leu	Arg	Gly	Val	Phe	Gly	Thr	Met	Leu	Asp	Gly	Glu	Gln			Asn	His	Thr	Leu	Arg	Gly	Val	Phe	Gly	Thr	Met	Leu	Asp	Gly	Glu	Gln				
				485																				490															495																		
Ala	Arg	Leu	Asn	Pro	Ala	Ser	Ala	Val	Phe	Asp	Ser	Thr	Ser	Arg	Ser					Ala	Arg	Leu	Asn	Pro	Ala	Ser	Ala	Val	Phe	Asp	Ser	Thr	Ser	Arg	Ser			Ala	Arg	Leu	Asn	Pro	Ala	Ser	Ala	Val	Phe	Asp	Ser	Thr	Ser	Arg	Ser				
				500																				505															510																		
Arg	Ile	Thr	Arg	Val	Ser	Ser	Ser	Ser	Ile	Lys	Ala	Ala	Tyr	Thr	Thr					Arg	Ile	Thr	Arg	Val	Ser	Ser	Ser	Ser	Ile	Lys	Ala	Ala	Tyr	Thr	Thr			Arg	Ile	Thr	Arg	Val	Ser	Ser	Ser	Ser	Ile	Lys	Ala	Ala	Tyr	Thr	Thr				
				515																				520															525																		
Ser	Thr	Cys	Phe	Lys	Val	Val	Lys	Thr	Asn	Lys	Thr	Tyr	Cys	Leu	Ser					Ser	Thr	Cys	Phe	Lys	Val	Val	Lys	Thr	Asn	Lys	Thr	Tyr	Cys	Leu	Ser			Ser	Thr	Cys	Phe	Lys	Val	Val	Lys	Thr	Asn	Lys	Thr	Tyr	Cys	Leu	Ser				
				530																				535															540																		
Ile	Ala	Glu	Ile	Ser	Asn	Thr	Leu	Phe	Gly	Glu	Phe	Arg	Ile	Val	Pro					Ile	Ala	Glu	Ile	Ser	Asn	Thr	Leu	Phe	Gly	Glu	Phe	Arg	Ile	Val	Pro			Ile	Ala	Glu	Ile	Ser	Asn	Thr	Leu	Phe	Gly	Glu	Phe	Arg	Ile	Val	Pro				
				545																				550															555																		
Leu	Leu	Val	Glu	Ile	Leu	Lys	Asp	Asp	Gly	Val	Arg	Glu	Ala	Arg	Ser					Leu	Leu	Val	Glu	Ile	Leu	Lys	Asp	Asp	Gly	Val	Arg	Glu	Ala	Arg	Ser			Leu	Leu	Val	Glu	Ile	Leu	Lys	Asp	Asp	Gly	Val	Arg	Glu	Ala	Arg	Ser				
				565																				570															575																		

Gly

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 57 base pairs
 (B) TYPE: nucleic acid

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(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:
(B) CLONE: 538-46.26 (Junction A)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CACATACGAT TTAGGTGACA CTATAGAATA CAAGCTTTAT ACCATTATAG ATACATT

(2) INFORMATION FOR SEQ ID NO:32:

57

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:
(B) CLONE: 538-46.26 (Junction B)

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 88..102
(D) OTHER INFORMATION: /codon_start= 88
/function= "Translational start of hybrid protein"
/product= "N-terminal peptide"
/number= 1
/standard_name= "Translation of synthetic DNA
sequence"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 103..108
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /partial
/codon_start= 103
/product= "NDV Heamagglutinin-Neuraminidase"
/evidence= EXPERIMENTAL
/gene= "HN"
/number= 2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

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CATGTAGTCG ACTCTAGAAA AAATTGAAAA ACTATTCTAA TTTATTGCAC GGAGATCTTT 60
TTTTTTTTTT TTTTTTTTGG CATATAAATG AATTCGGATC CG GAC CGC 108
Asp Arg
1

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Asp Arg
1

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:
(B) CLONE: 538-46.26 (Junction C)

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 70..84
(D) OTHER INFORMATION: /codon_start= 70
/function= "Translational start of hybrid protein"
/product= "N-terminal peptide"
/number= 1
/standard_name= "Translation of synthetic DNA
sequence"

- (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 85..108
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /partial
/codon_start= 85
/function= "marker enzyme"
/product= "Beta-galactosidase"
/evidence= EXPERIMENTAL
/gene= "lacZ"
/number= 2
/citation= ([1])

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(x) PUBLICATION INFORMATION:

- (A) AUTHORS: Ferrari, Franco A
Trach, Kathleen
Hoch, James A
(B) TITLE: Sequence Analysis of the spo0B Locus Reveals
a Polycistronic Transcription Unit
(C) JOURNAL: J. Bacteriol.
(D) VOLUME: 161
(E) ISSUE: 2
(F) PAGES: 556-562
(G) DATE: Feb.-1985

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
TGCGACATCA AGAATCAAAC CGAATGCCCT CGACTCTAGA ATTCATTTT GTTTTTTCT 60
ATGCTATAA ATG AAT TCG GAT CCC GTC GTT TTA CAA CGT CGT GAC TGG 108
Met Asn Ser Asp Pro Val Val Leu Gln Arg Arg Asp Trp
      1             5 1             5
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Met Asn Ser Asp Pro
 1             5
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Val Val Leu Gln Arg Arg Asp Trp
 1             5
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

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(A) ORGANISM: Plasmid

(vii) IMMEDIATE SOURCE:

(B) CLONE: 538-46.26

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..54

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /partial

/codon_start= 1

/function= "marker enzyme"

/product= "Beta-galactosidase"

/evidence= EXPERIMENTAL

/gene= "lacZ"

/number= 1

/citation= ([1])

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 55..63

(C) IDENTIFICATION METHOD: experimental

(D) OTHER INFORMATION: /codon_start= 55

/function= "Translational finish of hybrid protein"

/product= "C-terminal peptide"

/evidence= EXPERIMENTAL

/number= 2

/standard_name= "Translation of synthetic DNA sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GAA ATC CAG CTG AGC GCC GGT CGC TAC CAT TAC CAG TTG GTC TGG TGT	48
Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys	
1 5 10 15	
CAA AAA GAT CCA TAATTAATTA ACCCGGGTCG AGGTCGAAG ACCAAATCT	100
Gln Lys Asp Pro	
1	
AACATGGT	108

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys
1 5 10 15
Gln Lys

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(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asp Pro
1

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 57 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Plasmid

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: 538-46.26 (Junction E)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AGATCCCCGG GCGAGCTCGA ATTCGTAATC ATGCTCATAG CTGTTTCCTG TGTGAAA

57

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Pseudorabies virus \ Synthetic oligonucleotide primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CGCGAATTTCG CTCGCAGCGC TATTGGC

27

(2) INFORMATION FOR SEQ ID NO:42:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: N
- (iv) ANTI-SENSE: N
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Pseudorabies virus \ Synthetic oligonucleotide primer
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GTAGGAGTGG CTGCTGAAG

19

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What is claimed is:

- 5
1. A recombinant swinepox virus capable of replication in an animal into which the recombinant swinepox virus is introduced which comprises swinepox viral DNA and foreign DNA, encoding RNA which does not naturally
10 occur in the animal into which the recombinant swinepox virus is introduced, the foreign DNA being inserted into the swinepox viral DNA at an insertion site which is not essential for replication of the swinepox virus and being under the control of a
15 promoter.
 2. A recombinant swinepox virus of claim 1, wherein the RNA encodes a polypeptide.
 - 20 3. A recombinant swinepox virus of claim 2, wherein the polypeptide is antigenic in the animal.
 4. A recombinant swinepox virus of claim 1, wherein the insertion site is present within the larger HindIII to
25 BglII subfragment of the HindIII M fragment of the swinepox virus.
 5. A recombinant swinepox virus of claim 4, wherein the insertion site is within an open reading frame
30 contained in the HindIII to BglII subfragment.
 6. A recombinant swinepox virus of claim 5, wherein the insertion site is the AccI restriction endonuclease site located in the HindIII to BglII subfragment.
35
 7. A recombinant swinepox virus of claim 1, wherein the insertion site is within an open reading frame encoding swinepox thymidine kinase.

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8. A recombinant swinepox virus of claim 3, wherein the antigenic polypeptide is or is from PRV glycoprotein 50, PRV glycoprotein II, PRV glycoprotein III, PRV glycoprotein H, TGE glycoprotein 195, TGE matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hyodysenteriae* protective antigen, BVD glycoprotein 55, NDV hemagglutinin-neuraminidase, swine flu hemagglutinin and swine flu neuraminidase.
9. A recombinant swinepox virus of claim 3, wherein the antigenic polypeptide is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus, Swine Influenza Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*.
10. A recombinant swinepox virus of claim 8, wherein the antigenic polypeptide is PRV glycoprotein 50.
11. A recombinant swinepox virus of claim 8, wherein the antigenic polypeptide is NDV hemagglutinin-neuraminidase.
12. A recombinant swinepox virus of claim 2, wherein the polypeptide is a detectable marker.
13. A recombinant swinepox virus of claim 2, wherein the polypeptide is *E. coli* β -galactosidase.
14. A recombinant swinepox virus of claim 13, designated S-SPV-003 (ATCC Accession No. VR 2335).
15. A recombinant swinepox virus of claim 10 further comprising foreign DNA encoding a polypeptide which is a detectable marker.

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16. A recombinant swinepox virus of claim 15 designated S-SPV-008 (ATCC Accession No. VR 2339).
17. A recombinant swinepox virus of claim 11 further
5 comprising foreign DNA encoding a polypeptide which is a detectable marker.
18. A recombinant swinepox virus of claim 17, designated S-SPV-009 (ATCC Accession No. VR 2344).
- 10 19. A recombinant swinepox virus of claim 1, wherein the promoter is a swinepox viral promoter.
20. A recombinant swinepox virus of claim 1, wherein the
15 promoter is a synthetic pox viral promoter.
21. A homology vector for producing a recombinant swinepox virus by inserting foreign DNA into the genomic DNA of a swinepox virus which comprises a double-stranded DNA
20 molecule consisting essentially of:
 - a) double-stranded foreign DNA encoding RNA which
does not naturally occur in an animal into which
the recombinant swinepox virus is introduced;
25
 - b) at one end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at one side of a site on the genomic DNA which is not essential for replication of the
30 swinepox virus; and
 - c) at the other end of the foreign DNA, double-stranded swinepox viral DNA homologous to genomic DNA located at the other side of the same site on
35 the genomic DNA.

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22. A homology vector of claim 21, wherein the RNA encodes a polypeptide.
23. A homology vector of claim 22, wherein the polypeptide
5 is antigenic in the animal.
24. A homology vector of claim 23, wherein the antigenic polypeptide is or is from PRV glycoprotein 50, PRV glycoprotein II, PRV glycoprotein III, PRV
10 glycoprotein H, TGE glycoprotein 195, TGE matrix protein, swine rotavirus glycoprotein 38, swine parvovirus capsid protein, *Serpulina hyodysenteriae* protective antigen, BVD glycoprotein 55, NDV hemagglutinin-neuraminidase, swine flu hemagglutinin
15 or swine flu neuraminidase.
25. A homology vector of claim 23, wherein the antigenic polypeptide is or is from *Serpulina hyodysenteriae*, Foot and Mouth Disease Virus, Hog Cholera Virus, Swine
20 Influenza Virus, African Swine Fever Virus or *Mycoplasma hyopneumoniae*.
26. A homology vector of claim 22, wherein the polypeptide is a detectable marker.
25
27. A homology vector of claim 22, wherein the polypeptide is *E. coli* β -galactosidase.
28. A homology vector of claim 21, wherein the double-
30 stranded swinepox viral DNA is homologous to genomic DNA present within the larger HindIII to BglII subfragment of the HindIII M fragment of swinepox virus.
- 35 29. A homology vector of claim 28, wherein the double-stranded swinepox viral DNA is homologous to genomic

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DNA present within the open reading frame contained in the HindIII to BglII subfragment.

30. A homology vector of claim 29, wherein the double-stranded swinepox viral DNA is homologous to genomic DNA present within the AccI restriction endonuclease site located in the HindIII to BglII subfragment.
31. A homology vector of claim 21, wherein the double-stranded swinepox viral DNA is homologous to genomic DNA present within the open reading frame encoding swinepox thymidine kinase.
32. A homology vector of claim 21, wherein the foreign DNA further comprises a synthetic pox viral promoter.
33. A vaccine which comprises an effective immunizing amount of the recombinant swinepox virus of claims 3, 8, 9, 16 or 18 and a suitable carrier.
34. A vaccine which comprises an effective immunizing amount of the recombinant swinepox virus of claim 16 and a suitable carrier.
35. A method of immunizing an animal which comprises administering to the animal an effective immunizing dose of the vaccine of claim 33.
36. A method of immunizing an animal which comprises administering to the animal an effective immunizing dose of the vaccine of claim 34.
37. A method of claim 35 or 36, wherein the animal is a swine, bovine equine, caprine, or ovine.
38. A method for testing a swine to determine whether the swine has been vaccinated with the vaccine of claim 33

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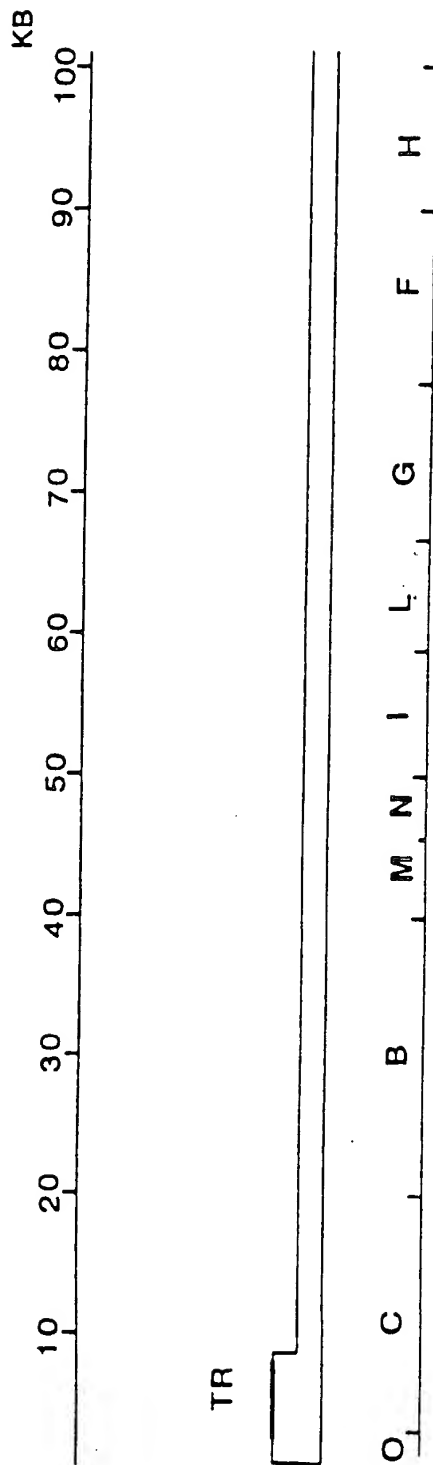
or is infected with a naturally-occurring pseudorabies virus which comprises:

- 5 a) obtaining from the swine to be tested
 a sample of a suitable body fluid;
- 10 b) detecting in the sample the presence
 of antibodies to pseudorabies virus,
 the absence of such antibodies
 indicating that the swine has been
 neither vaccinated nor infected; and
- 15 c) for the swine in which antibodies to
 pseudorabies virus are present,
 detecting in the sample the absence of
 antibodies to pseudorabies virus
 antigens which are normally present in
 the body fluid of a swine infected by
 the naturally-occurring pseudorabies
20 virus but which are not present in a
 vaccinated swine, the absence of such
 antibodies indicating that the swine
 was vaccinated and is not infected.
- 25 39. A host cell infected with the recombinant swinepox
 virus of claim 1.
- 30 40. A host cell of claim 39, wherein the host cell is a
 mammalian cell.
41. A host cell of claim 40, wherein the mammalian cell is
 a Vero cell.
- 35 42. A host cell of claim 41, wherein the mammalian cell is
 an EMSK cell.

1/20

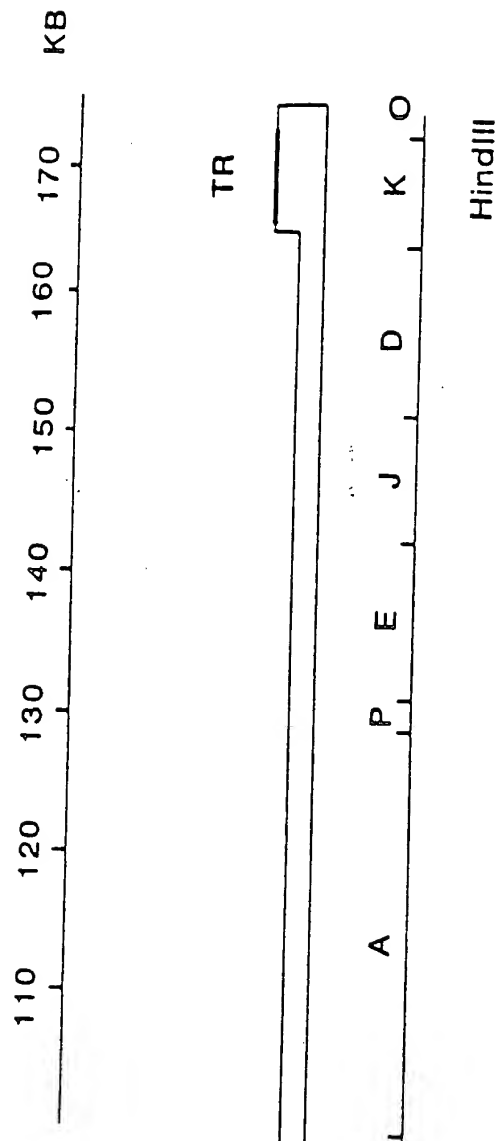
FIGURE 1A

1/20 FIGURE 1A	2/20 FIGURE 1B
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2/20

FIGURE 1B



3/20

3/20 FIGURE 2A	4/20 FIGURE 2B
-------------------	-------------------

FIGURE 2A

AA GTATCCAGAGTTGTTGAATGCCCTTATCGTACCTAATAATAATAGAGTTATAACT
GAATAAGTATATATAAATGATTGTTTTTATTAATGTTTATCGCATTTAGTTTGTGCTGT
ATGGTTATCATATACATTTTAAAGCCGCTATATGATAAATGAAAATATAAAGCACTTAT
TTTTGTTAGTATAATAACAAATGCCGTCGTATATGTATCCGAAGAACGCAAGAAAAGTA
ATTTCAAAGATTATATCATTAACAACCTTGATATTAAAAAATCTCCCTAAAAAATATATAAAT
ACCATGTTAGAAATTTGGTCTACATGGAAATCTACCAGCTTGTATGTATAAAGATGCCGTA
MetProSer.....
TCATATGATATAAATAATAAGATTTTTACCTTATAATTTGTGTATGGTTAAAGATTTA
ATAAATGTTATAAAATCATCATCTGTAATAGATAGTACATCAATCAATCTGTATTAAAA
CATCGTAGAGCGTTAATAGATTACGGCGATCAAGACATTATCACTTTAATGATCATTAAT
AAGTTACTATCGATAGATGATATATCCCTATATATAGATAAAAAAATAATTCATGTAAAC
.....IleHisVal

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FIGURE 2B

GAGATATTAAATCATGTAAATGCTCGATATGTTCCGACTCTATAACACATCATATATATG
 Asp11eLysSer.....
 AAACAACATCATGTATAAATTATAAATCTACCGATAATGATCTTATGATAGTATTGTTCA
 ATCTAACTAGATATTTAATGCGATGGGATGATACATCCTAATCTTATAAGCGTAAAGGAT
 GGGTCCCCCTTATTGGATTATTACCGGTGATATAGGTATTAAATTTAAACTATATTCCA
 CCATGAATATAAATGGGCTACGGTATGGAGATATTACGTTATCTTCATACGATATGAGTA
 ATAAATTAGTCTCTATTATTAAATACACCCATATATGAGTTAATACCGTTTACTACATGTT
 GTTCACTCAATGAATATTATTCAAAATTTGTGATTTTAATAAATGTTATTTTAGAATATA
 TGATATCTATTATATATAGAAATATTGATCGTAAAGATTTAATAACATTAAAGAAAT
 TTATTTCAAAAGTCGTAATACTGTACTAGAAATCATCAGGCATATATTTTGTGATGCG
 GTGTACATGAACAAATTGAAATTGGAATAGATGAGCTCATTTAATGATGCTATGCCCCTG
 TACAGCTTATGCAATTTACTTCTAAAGGTAGCTACCATTAATATTAGAGGAAATCAAAGAA
 LysGluI
 TATAACGTAATTTTCTTTTAAATAAAATAAAATACTTTTTTTAAACAAGGGTGCT
 le---
 ACCTTGCTAAATGTATCTTGTATTTTGGATCTGATGCAAGATTATTAATAATCGTATG
 AAAAGTAGTAGATATAGTTTATATCGTTACTGGACATGATATATGTTTAGTTAATTCT
 TCTTTGGCATGAATTCTACACGTCGGANAAGGTAATGTATCTATAATGGTATAAAGCTT

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5/20	FIGURE 3A
6/20	FIGURE 3B
7/20	FIGURE 3C

FIGURE 3A

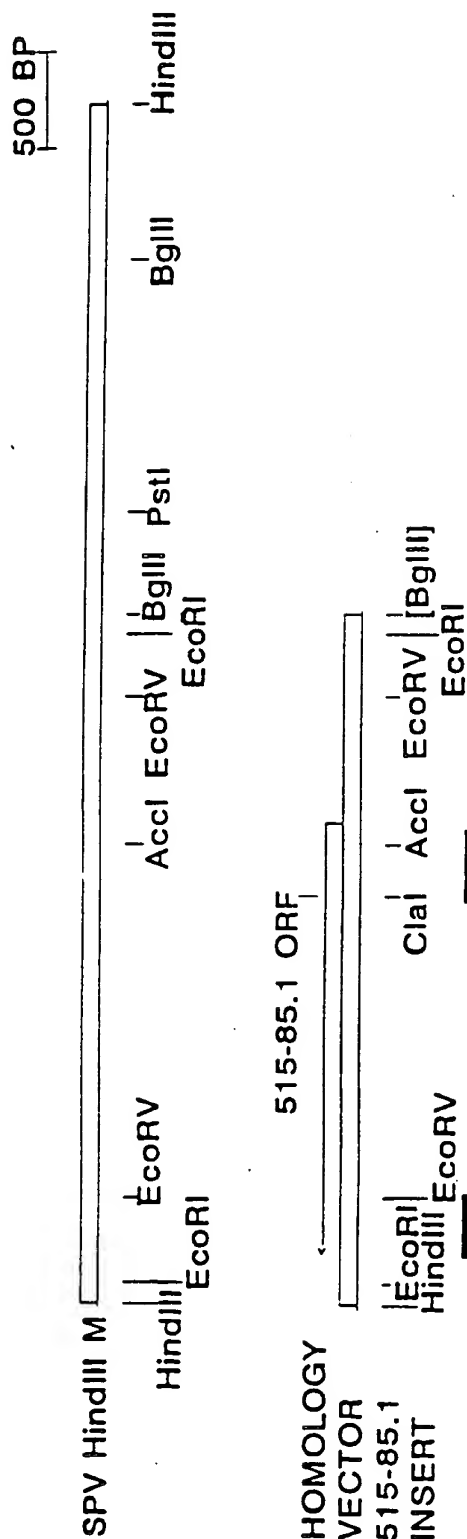


FIGURE 3B

	10	20	30	40	50	60	70
(A) VV	MFMYPEFARKALSKLISKKLNIEKVSSKHQLVLVDYGLHGLLPKSLYLEAINSDILNVRRFFPPEIINVT	*	*	*	*	*	*
orf O1L	::: :::				:	:	:
(B) SPV	MPSMYYPKNARKVISKIISLQLDIKKLPKKYINTMLEFGLHGNLPACMYKDVAVSVDINNIRFLPYNCVMVK						
AccI-ClaI							

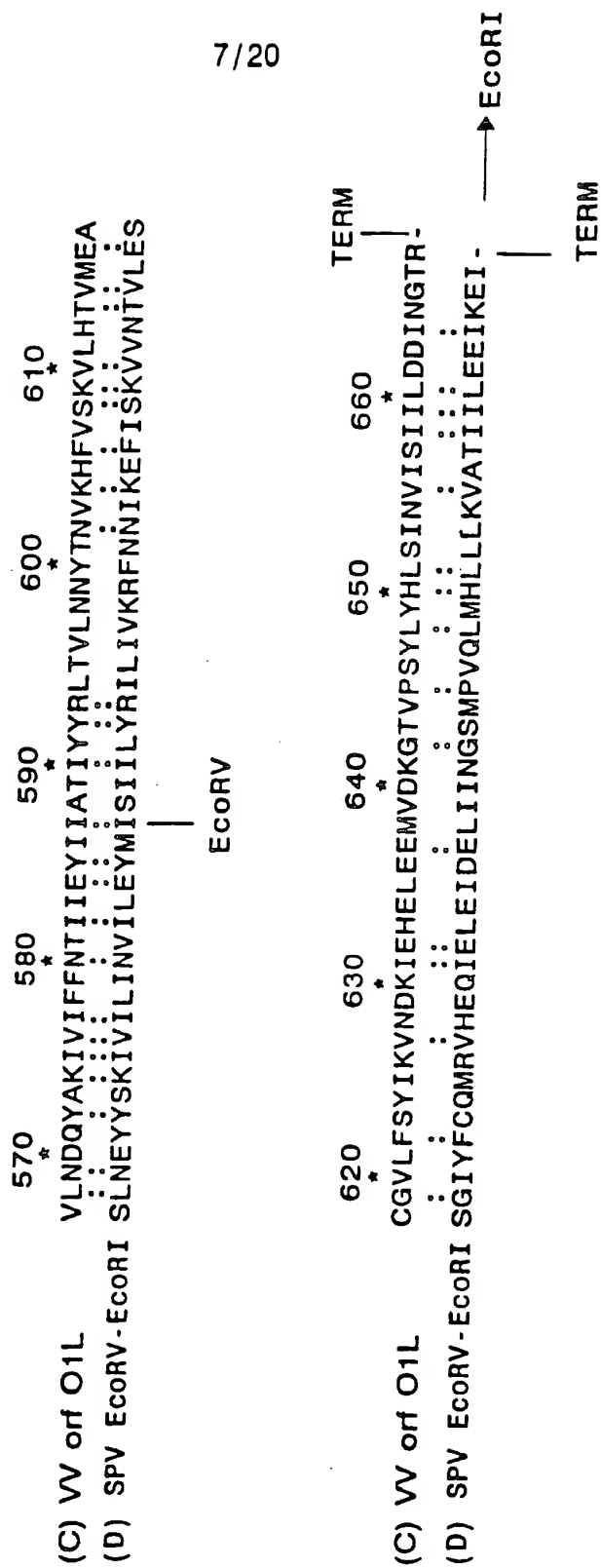
ACCI

	80	90	100	110	120
	*	*	*	*	*
(A) VV	DIVKALQNSCRVDEYLKAVSLYHKNSLMVSGPNVVK - LMIEYNLLTHSDLEWLINENVVKA				
orf 01L	:	:	:	:	:
(B) SPV	DLINVIKSSVIDTRLHQSVLKHRRALIDYGQDIIITLMIINKLLSIDDISYILDKKIIHV				
AccI-ClaI					
					ClaI

Clai

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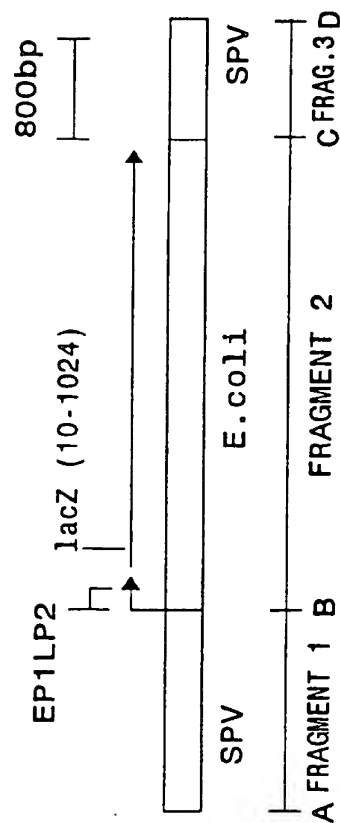
FIGURE 3C



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8/20 FIGURE 4A
9/20 FIGURE 4B
10/20 FIGURE 4C

FIGURE 4A



<u>DNA</u>	<u>ORIGIN</u>	<u>SITES</u>	<u>SIZE</u>
VECTOR	pSP64	HindIII - BamHI	~2972 BP
FRAGMENT 1	SPV HindIII	M HindIII - AccI	~2015 BP
FRAGMENT 2	pJF751	BamHI - PvuII	~3010 BP
FRAGMENT 3	SPV HindIII	M AccI - BglII	~1103 BP

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FIGURE 4B

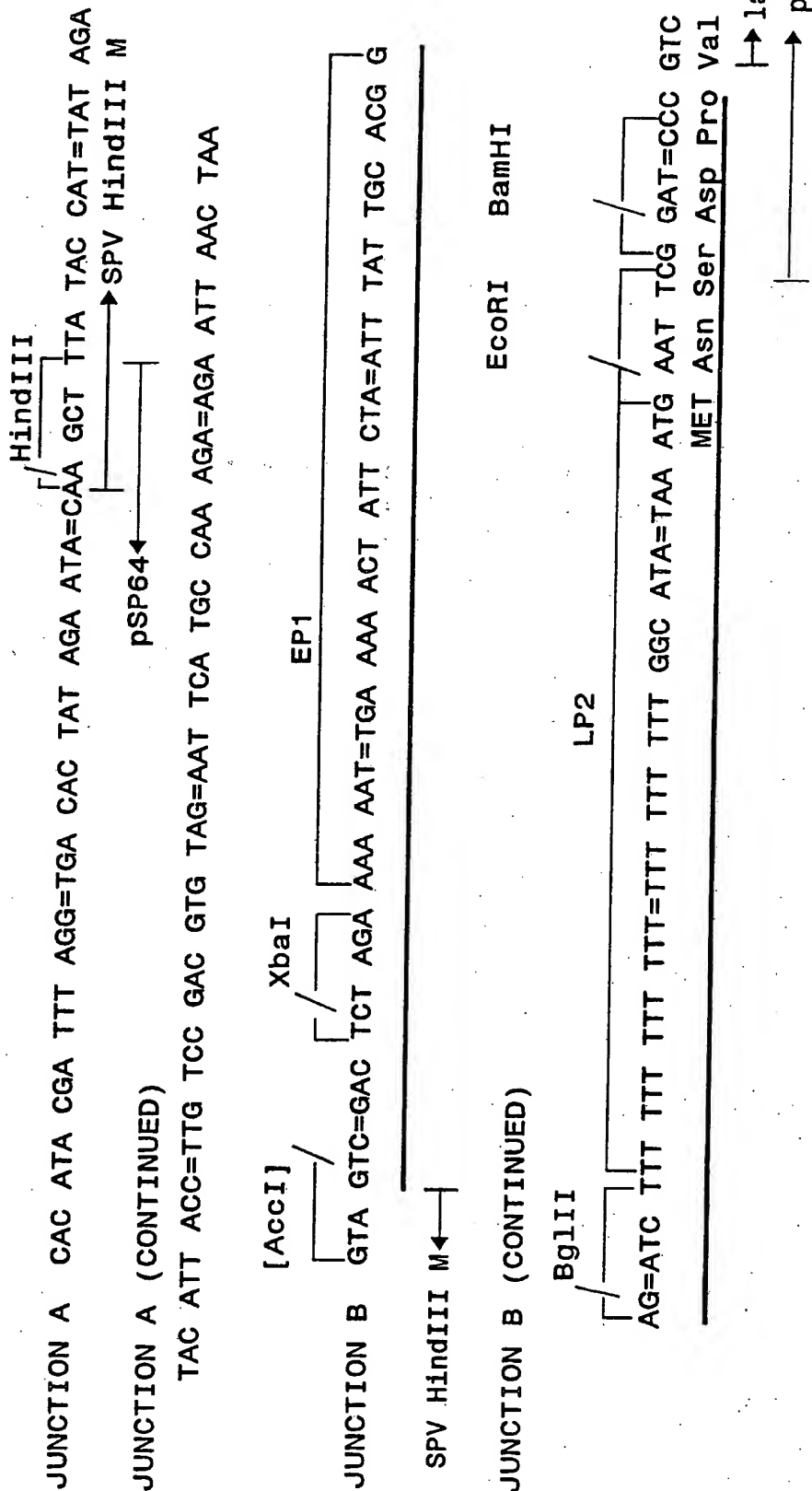
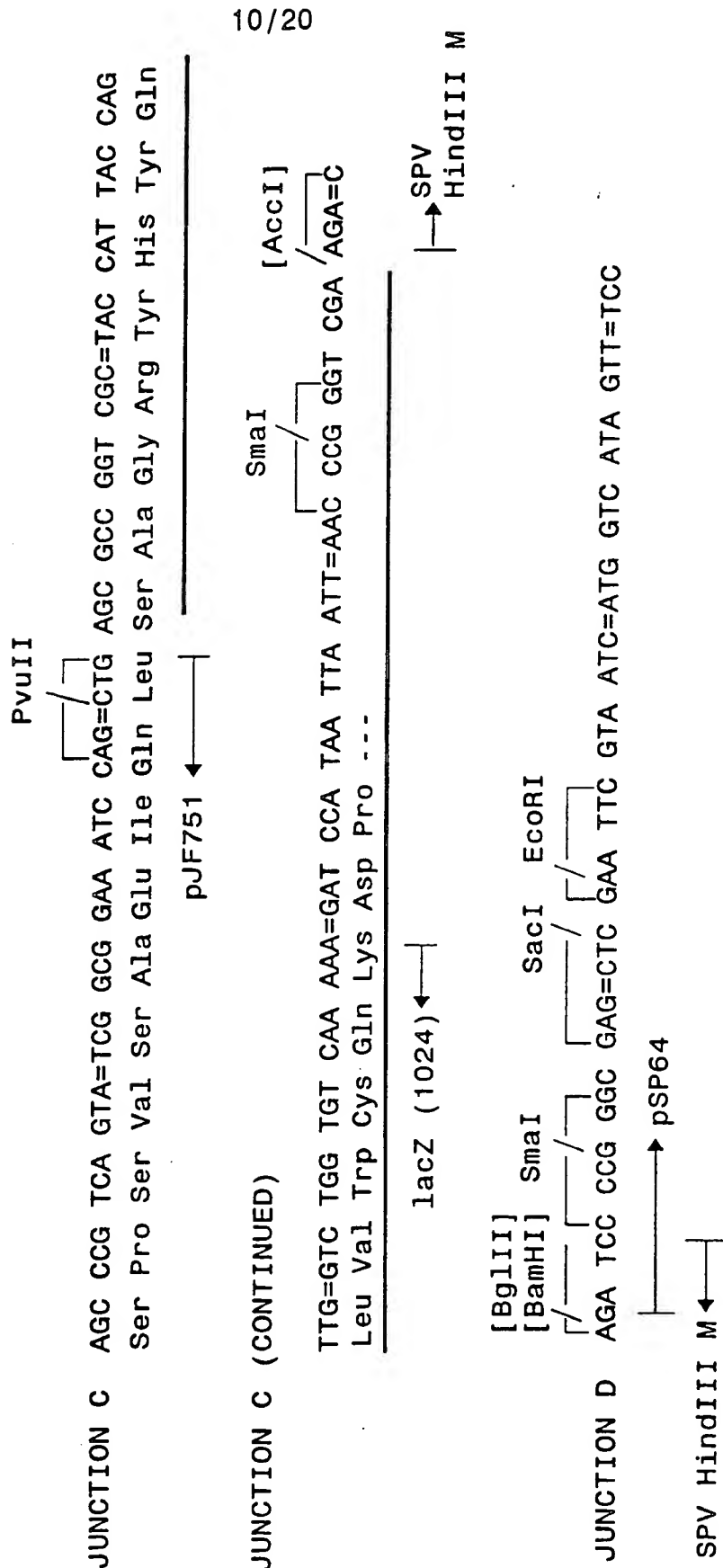


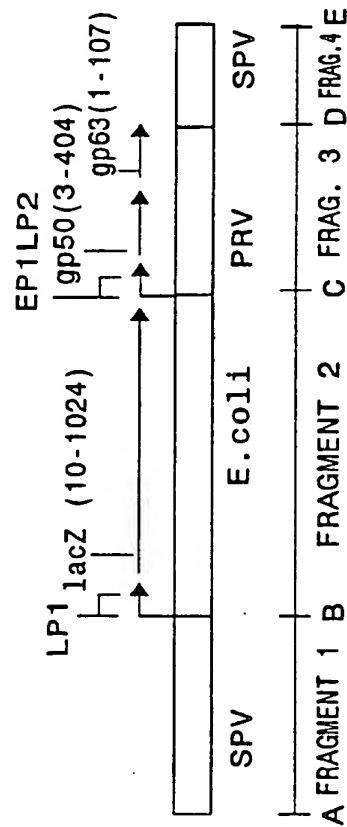
FIGURE 4C



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FIGURE 5A

11/20 FIGURE 5A
12/20 FIGURE 5B
13/20 FIGURE 5C



DNA	ORIGIN	SITES	SIZE
VECTOR	pSP64	HindIII-BamHI	~2972 BP
FRAGMENT 1	SPV HindIII	M HindIII-AccI	~2015 BP
FRAGMENT 2	pJF751	BamHI-PvuII	~3010 BP
FRAGMENT 3	PRV BamHI #7	EcoRI*-StuI	~1558 BP
FRAGMENT 4	SPV HindIII	M AccI-BglII	~1103 BP

* INTRODUCED VIA CLONING

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FIGURE 5B

JUNCTION A CAC ATA CGA TTT AGG=TGA CAC TAT AGA ATA=CAA GCT TTA TAC CAT=TAT AGA TAC ATT
 HindIII
 pSP64
 SPV HindIII M

JUNCTION B AAG CTG GTA GAT TTC=CAT GTA GGG CCG CCT=GCA GGT CGA CTC TAG=A
 [AccI] PstI SalI XbaI
 SPV HindIII M

JUNCTION B (CONTINUED)
 LP1 EcoRI BamHI
 AT TTC ATT TTG TTT=TTT TCT ATG CTA TAA=ATG AAT TCG GAT CCC=GTC GTT TTA CAA
 MET Asn Ser Asp Pro Val Leu Gln
 lacZ(10)
 pJF751

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FIGURE 5C

PvuII

JUNCTION C GTA TCG GCG GAA ATC CAG CTG AGC=GCC GGT CGC TAC CAT=TAC CAG TTG GTC TGG=TGT
 Val Ser Ala Glu Ile Gln Leu Ser Ala Gly Arg Tyr His Tyr Gln Leu Val Trp Cys

pJF751

PstI SalI

CAA=AAA GAT CCA TAA TTA=ATT AAC CCG GCC GCC=TGC AGG TGG ACT
 Gln Lys Asp Pro ---

←
 lacZ (1024)

XbaI

EP1

BglII

CT AGA AAA AAT TGA=AAA ACT ATT CTA ATT=TAT TGC ACG GAG ATC=†

EcoRI

LP2

TT TTT TTT TTT=TTT TTT GGC ATA TAA=ATG AAT TCG CTC GCA=GCG CTA TTG GCG GCG
 MET Asn Ser Leu Ala Ala Leu Leu Ala Ala

gp50(3)

PRV BamHI #7

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FIGURE 5D

JUNCTION D CGC GTG CAC CAC GAG=GGA CTC TAG AGG ATC=CAT AAT TAA TTA ATT=AA
 Arg Val His His Glu

gp63(107)←

PRV BamHI #7←

[StuI] XbaI BamHI

JUNCTION D (CONTINUED)

ETTS SmaI Sali PstI Sali PstI [AccI]
 T TTT TAT CCC GGG=TCG ACC TGC AGG CCG=CCG GGT CGA CCT GCA=GGC GGC CAG AC

SPV
HindIII M

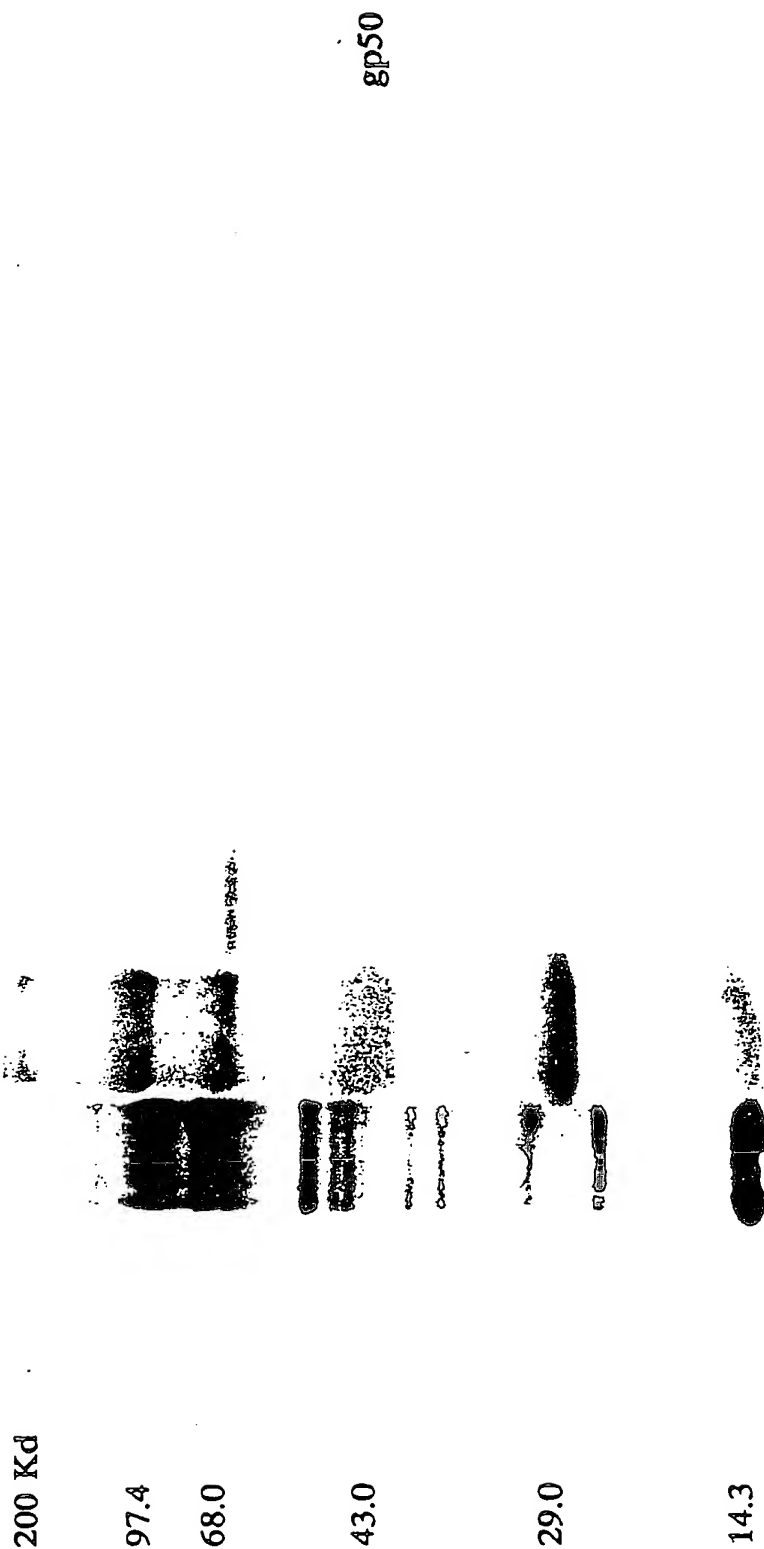
[BglII], [BamHI] SacI EcoRI

JUNCTION E AGA TCC CCG GGC GAG=CTC GAA TTC GTA ATC=ATG GTC ATA GCT GTT=TCC TGT GTG AAA
 SPV
HindIII M

← pSP64

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FIGURE 6
A B C D E F G



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Figure 7

ACGGGTAGAACGGTAAGAGAGGGCCGCCCTCAATTGCGAGCCAGACTTCACAACCTCCGT

AvaII

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TCTACCGCTTCACCGACAACAGTCCTCAATCATGGACCGCGCGCTTAGCCAAGTTGCGTT

MetAspGly.....

AGAGAATGATGAAAGAGAGGGCAAAAATACATGGCGCTTGATATTCGGATTGCAATCTT
ATTCTTAACAGTAGTGACCTTGGCTATATCTGTAGCCTCCCTTTTATATAGCATGGGGGC
TAGCACACCTAGCGATCTTGTAGGCATACCGACTAGGATTTCCAGGGCAGAAGAAAAGAT
TACATCTACACTTGGTTCCAATCAAGATGTAGTAGATAGGATATATAAGCAAGTGGCCCT
TGAGTCTCCATTGGCATTGTTAAATACTGAGACCACAATTATGAACGCAATAACATCTCT
CTCTTATCAGATTAATGGAGCTGCAAACAACAGCGGGTGGGGGGCACCTATTCATGACCC
AGATTATATAGGGGGGATAGGCAAAGAAGCTCATTGTAGATGATGCTAGTGATGTCACATC
ATTCTATCCCTCTGCATTTCAAGAACATCTGAATTTTATCCCGGCGCCTACTACAGGATC
AGGTTGCACTCGAATACCCTCATTTGACATGAGTGCTACCCATTACTGCTACACCCATAA
TGTAATATTGTCTGGATGCAGAGATCACTCACACTCACATCAGTATTTAGCACTTGGTGT
GCTCCGGACATCTGCAACAGGGAGGGTATTCTTTTCTACTCTGCGTTCCATCAACCTGGA
CGACACCCAAAATCGGAAGTCTTGCAAGTGTGAGTGCAACTCCCCTGGGTTGTGATATGCT
GTGCTCGAAAGCCACGGAGACAGAGGAAGAAGATTATAACTCAGCTGTCCCTACGCGGAT
GGTACATGGGAGGTTAGGTTTCGACGGCCAATATCACGAAAAGGACCTAGATGTCACAAC
ATTATTCGGGGACTGGGTGGCCAATACCCAGGAGTAGGGGGTGGATCTTTTATTGACAG
CCGCGTGTGGTTCTCAGTCTACGGAGGGTTAAAAACCAATACACCCAGTGACACTGTACA
GGAAGGGAAATATGTGATATACAAGCGATACAATGACACATGCCAGATGAGCAAGACTA
CCAGATTCTGAATGGCCAAGTCTTCGTATAAGCCTGGACGGTTTGGTGGGAAACGCATACA
GCAGGCTATCTTATCTATCAAAGTGTCAACATCCTTAGGGCAAGACCCGGTACTGACTGT
ACCGCCCAACACAGTCACACTCATGGGGCCGAAGGCAGAATTCTCACAGTAGGGACATC
CCATTTCTTGATCAGCGAGGGTCATCATACTTCTCTCCCGCGTTATTATATCCTATGAC
AGTCAGCAACAAAACAGCCAATCTTCATAGTCCTTATACATTCAATGCCTTCACTCGGCC
AGGTAGTATCCCTTGCCAGGCTTCAGCAAGATGCCCCAACTCATGTGTACTGGAGTCTA
TACAGATCCATATCCCCTAATCTTCTATAGAAAACACACCTTGGCAGGGGTATTTCGGGAC
AATGCTTGATGGTGAACAAGCAAGACTTAACCCTGCGTCTGCAGTATTCGATAGCACATC
CCGCAGTCGCATAACTCGAGTGAGTTCAAGCAGCATCAAAGCAGCATACACAACATCAAC
TTGTTTTAAAGTGGTCAAGACCAATAAGACCTATTGTCTCAGCATTGCTGAAATATCTAA
TACTCTCTTCGGAGAATTCAGAATCGTCCCGTTACTAGTTGAGATCCTCAAAGATGACGG
GGTTAGAGAAGCCAGGTCTGGCTAGTTGAGTCAACTATGAAAGAGTTGGAAAGATGGCAT
.....ArgSerGly---

NaeI

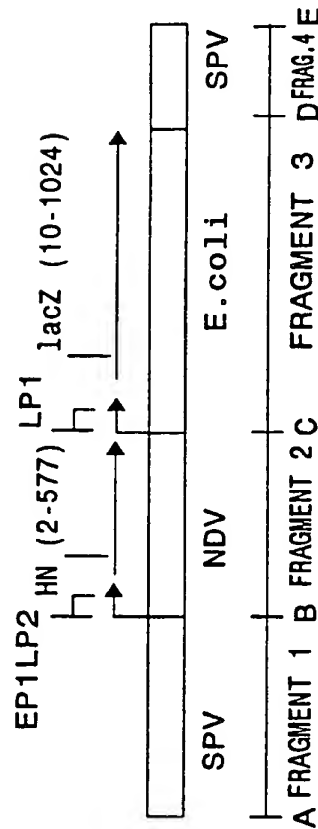
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TGTATCACCTATCTTCTGCGACATCAAGAATCAAACCGAATGCCGGC

FIGURE 8A

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17/20 FIGURE 8A
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19/20 FIGURE 8C
20/20 FIGURE 8D

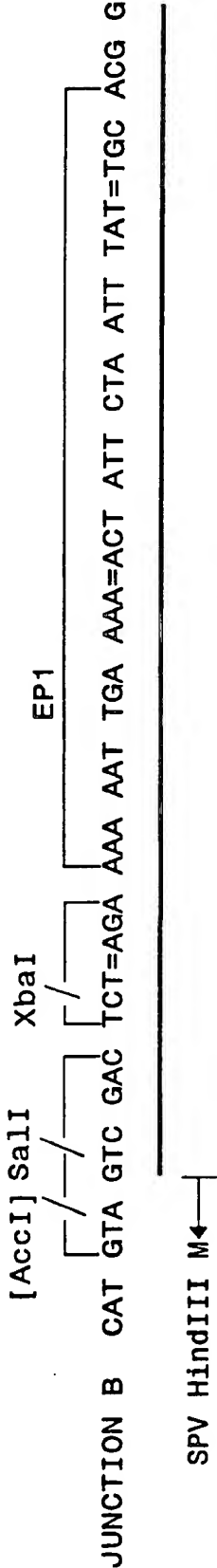


<u>DNA</u>	<u>ORIGIN</u>	<u>SITES</u>	<u>SIZE</u>
VECTOR	pSP64	HindIII - BamHI	~2972 BP
FRAGMENT 1	SPV HindIII	M HindIII - AccI	~2015 BP
FRAGMENT 2	MDV HN cDNA	AvaII - NaeI	~1810 BP
FRAGMENT 3	pJF751	BamHI - PvuII	~3010 BP
FRAGMENT 4	SPV HindIII	M AccI - BglII	~1103 BP

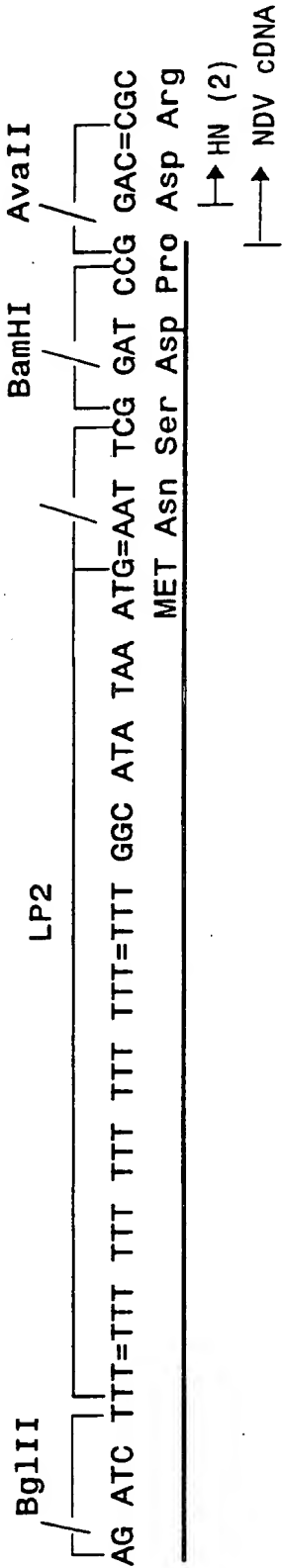
FIGURE 8B

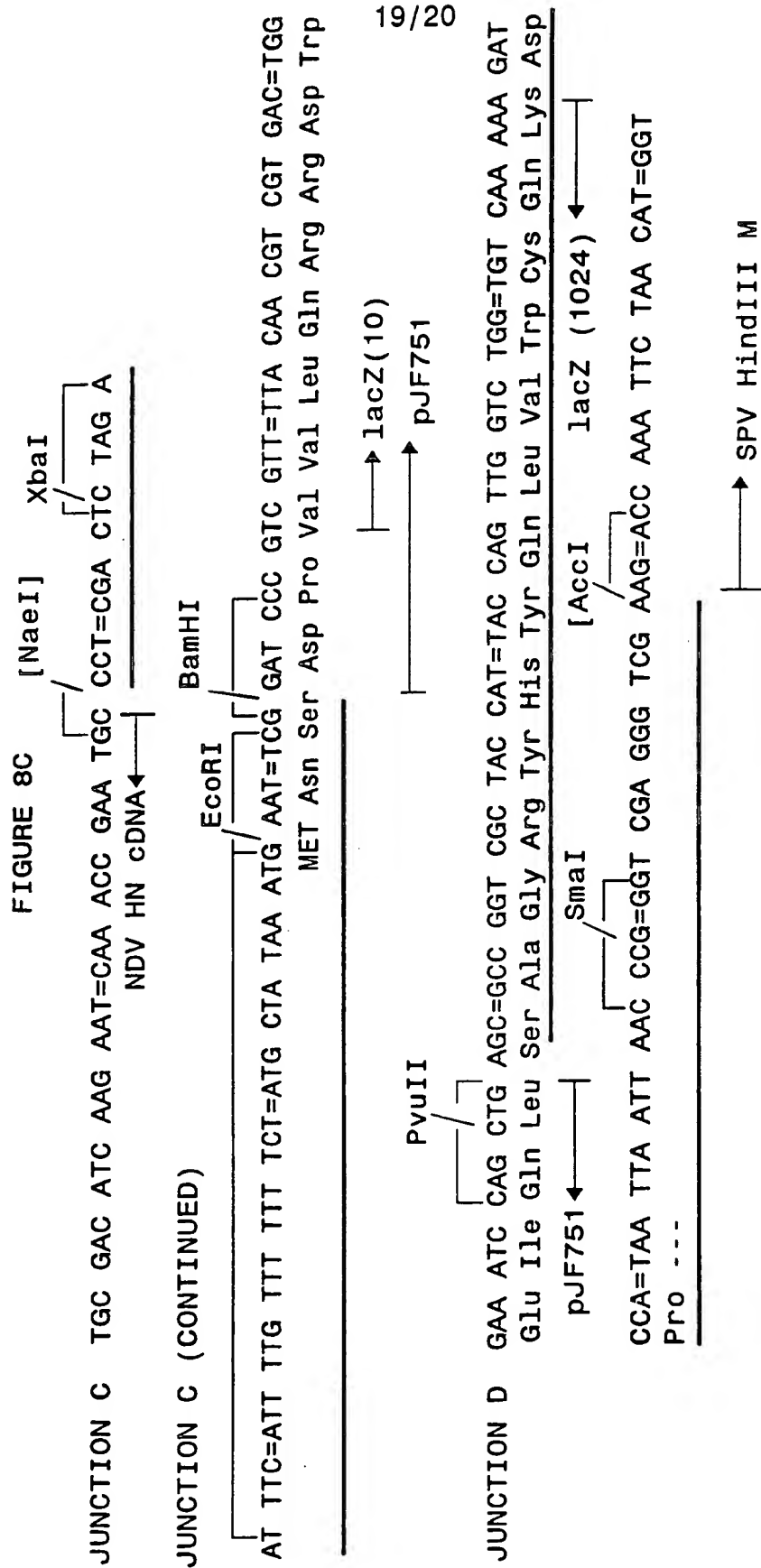


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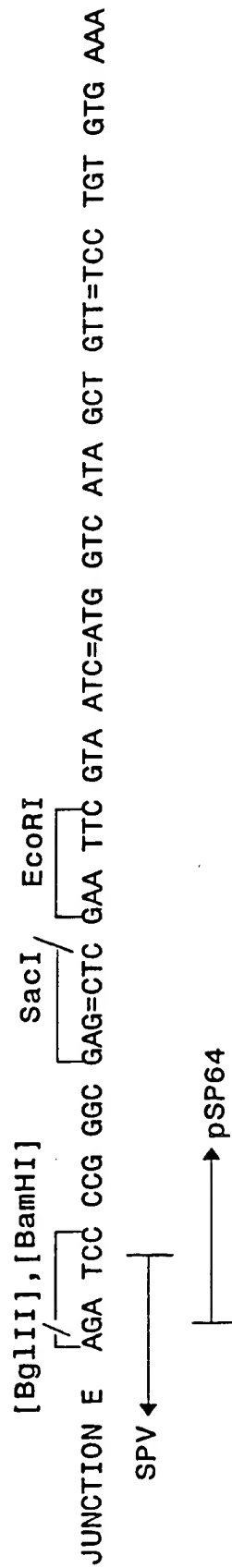
JUNCTION B (CONTINUED)





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FIGURE 8D



INTERNATIONAL SEARCH REPORT

Int. national application No.

PCT/US93/00324

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : C12N 7/00, 5/00, 15/00; C12Q 1/00; A61K 39/12

US CL : 435/7.1, 235.1, 240.2; 424/89

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 235.1, 240.2; 424/89

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Virology, Volume 180, issued January 1991, R.F. Massung et al., "The Molecular Biology of Swinepox Virus, II. The Infectious Cycle", pages 355-364, see entire document.	1-42
Y	Journal of Virological Methods, Volume 20, issued March 1988, W.M. Schnitzlein, "A Rapid Method for Identifying the Thymidine Kinase Genes of Avipoxviruses", pages 341-352, see entire document.	1-42
Y	EP, A, 0,261,940, (Panicali et al.) 30 March 1988, see entire document, especially p. 6, lines 19-63.	1-42



Further documents are listed in the continuation of Box C.



See patent family annex.

A	document defining the general state of the art which is not considered to be part of particular relevance	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E	earlier document published on or after the international filing date	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means	*Z*	document member of the same patent family
P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

09 APRIL 1993

Date of mailing of the international search report

21 APR 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

JOAN ELLIS

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00324

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	Journal of Virology, Volume 66, No. 5, issued May 1992, B.G. Klupp et al., "Identification and Characterization of Pseudorabies Virus Glycoprotein H", pages 3048-3055, see entire document.	8, 24, 33-42
Y	Journal of Virology, Volume 59, No. 3, issued September 1986, A.K. Robbins et al., "Pseudorabies Virus Encoding Glycoprotein gIII Is Not Essential for Growth in Tissue Culture", pages 635-645, see entire document.	8, 24, 33-42
Y	WO, A, 89/10965 (Petrovskis et al.) 16 November 1989, see entire document.	8, 24, 33-42
Y	Journal of Virology, Volume 61, No. 12, issued December 1987, C.C. Marchioli et al. "Evaluation of Pseudorabies Virus Glycoprotein gp50 as a Vaccine for Aujeszky's Disease in Mice and Swine: Expression by Vaccinia Virus and Chinese Hamster Ovary Cells", pages 3977-3982, see entire document.	8, 10, 24, 33-42
Y	Virology, Volume 151, issued January 1986, P.A. Kapke, "Sequence Analysis of the Porcine Transmissible Gastroenteritis Coronavirus Nucleocapsid Protein Gene", pages 41-49, see entire document.	8, 24, 33-37, 39-42
Y	Virology, Volume 165, issued March 1988, P.A. Kapke et al., "The Amino-Terminal Signal Peptide on the Porcine Transmissible Gastroenteritis Coronavirus Matrix Protein Is Not an Absolute Requirement for Membrane Translocation and Glycosylation" pages 367-376, see entire document.	8, 24, 33-37, 39-42
Y	WO, A, 88/02026 (Mazzara et al.) 24 March 1988, see entire document.	8, 24, 33-37, 39-42
Y	Journal of General Virology, Volume 69, issued May 1988, W.L. Mengeling et al., "Size and Antigenic Comparisons Among the Structural Proteins of Selected Autonomous Parvoviruses", pages 825-837, see entire document.	8, 24, 33-37, 39-42
Y	Virology, Volume 185, issued April 1991, F. Cosset et al., "Newcastle Disease Virus (NDV) Vaccine Based on Immunization with Avian Cells Expressing the NDV Hemagglutinin-Neuraminidase Glycoprotein", pages 862-866, see entire document.	8, 11, 24, 33-37, 39-42

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00324

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Immunology, Volume 73, issued March 1991, M.J. Francis et al., "Immunological Evaluation of the Multiple Antigen Peptide (MAP) System Using the Major Immunogenic Site of Foot-and-Mouuth Disease Virus", pages 249-254, see entire document.	9, 25, 33-37, 39-42
Y	Vaccine, Volume 8, issued February 1990, M. Agterberg et al., "Outer Membrane PhoE Protein of <u>Escherichia coli</u> as a Carrier for Foreign Antigenic Determinants: Immunogenicity of Epitopes of Foot-and-Mouth Disease Virus", pages 85-91, see entire document.	9, 25, 33-37, 39-42
Y	Infection and Immunity, Volume 49, No. 2, issued August 1985, M. Klinkert et al., "Surface Proteins of <u>Mycoplasma hyopneumoniae</u> Identified from an <u>Escherichia coli</u> Expression Plasmid Library", pages 329-335, see entire document.	9, 25, 33-37, 39-42
Y	Virology, Volume 142, issued June 1985, T.C. Whyard et al., "Monoclonal Antibodies Against African Swine Fever Viral Antigens", pages 416-420, see entire document.	9, 25, 33-37, 39-42
Y	Virology, Volume 183, issued July 1991, J.A. Feller et al., "Isolation and Molecular Characterization of the African Swinepox Virus Thymidine Kinase Gene", pages 578-585, see entire document.	1-42
Y	J. Sambrook et al., "Molecular Cloning, A Laboratory Manual", Second Edition, published 1989 by Cold Spring Harbor Laboratory Press (N.Y.), see pages 12.2-12.44.	1-42
Y	Biochimica et Biophysica Acta, Volume 1090, issued March 1991, A.L. Meyer et al., "Cloning and Sequence of an Infectious Bovine Rhinotracheitis Virus (BHV-1) Gene Homologous to Glycoprotein H of Herpes Simplex Virus" pages 267-269, see entire document.	8, 24, 33-37, 39-42

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/00324

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

CAS ONLINE, search terms: swinepox virus, pseudorabies, parvovirus serpulina hyodysenteriae, swine rotavirus, swine parvovirus capsid, swine influenza virus, foot and mouth disease virus, mycoplasma hyopneumoniae, hog cholera virus, African swine fever virus